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Relationship Between Sedentary Time and Physical Activity With Glycemic Variability and Oxidative Stress

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RELATIONSHIP BETWEEN SEDENTARY TIME AND PHYSICAL ACTIVITY WITH GLYCEMIC VARIABILITY AND OXIDATIVE STRESS

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DEDICATION

I would like to dedicate this dissertation to my canine companion, Amber Tech Sparks. Without her by my side I truly believe I would not have succeeded in this endeavor.

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The past 6 years have truly been a journey that required help from all the people in my life. There are too many to individually recognize, but a select few that deserve acknowledgement. First and foremost, I would like to express my deepest and sincerest gratitude to Dr. Xuewen Wang, my primary mentor, who accepted a kid from Louisiana into her lab and helped pave the way for the success I have today. Without her advising and continual support none of this would be possible. To my teaching mentor, Dr. J. Larry Durstine, whose guidance and compassion for instruction of students is second to none. To my dissertation committee members, Dr. Mark Sarzynski, Dr. J. Mark Davis, and Dr. Peter Grandjean, without their guidance throughout my PhD and during the dissertation process this would have been unfathomable.

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ABSTRACT

Overweight and obesity is becoming more prevalent in adults across the United States. Overweight or obese adults are at an increased risk for the development of cardiometabolic disorders. Glycemic variability has recently been introduced as a sensitive measurement of glycemic health as it incorporates oscillations in glucose concentrations over an extended period. Further, evidence has suggested that glycemic variability plays a pivotal role in the induction of oxidative stress commonly found in adults diagnosed with cardiometabolic disorders. Physical activity (PA) and exercise have been utilized as therapeutic treatments for overweight and obesity related complications. Therefore, the overall goal of this dissertation was to investigate the relationship between PA with glycemic variability and oxidative stress, and to examine the influence of exercise training on glycemic variability and oxidative stress in non-diabetic overweight or obese adults.

Three studies were conducted utilizing different designs to (study 1) inspect the current findings linking the relationship between sedentary behavior and physical activity (PA) with, and the influence of exercise on, continuous glucose monitor (CGM) assessed glycemic variability, (study 2; n=28) examine the cross-sectional relationship between objectively measured sedentary time and PA with glycemic variability and oxidative stress, and (study 3; n=8) evaluate the impact of a 12-week aerobic exercise intervention on glycemic variability and oxidative stress. The first study was a critical review of the

literature, while the second study utilized baseline data collected from the Weight Outlooks by Restriction of Diet and Sleep (WORDS) and the Aerobic Treadmill Exercise and Metabolism (A-TEAM) studies, and the third study utilized data collected for individuals that completed the A-TEAM study. For study two, objective assessment of sedentary time and PA was performed, while for study two and three glucose concentrations and glycemic variability were assessed via CGM and oxidative stress assessed as nitric oxide and myeloperoxidase concentrations in human serum (WORDS) or plasma (A-TEAM).

The first study found that a relationship between sedentary behavior and PA with glycemic control and glycemic variability exists in non-diabetic and diabetic adults. However, there were differential findings when examining the effect of a single bout of exercise or repeated bouts of exercise on glycemic control and glycemic variability between populations, while exercise training improved glycemic control and glycemic variability in type 2 diabetic adults. In the second study, a relationship was observed between PA minutes and energy expenditure of varying intensities with glucose concentrations, but not glycemic variability, and myeloperoxidase concentration, while fasting glucose concentration correlated with nitric oxide and myeloperoxidase concentrations, and the oxidative stress ratio in non-diabetic overweight or obese adults. The third study found that, although myeloperoxidase concentration decreased and the oxidative stress ratio improved, glucose concentrations and glycemic variability did not change following exercise training in non-diabetic overweight or obese adults. Yet, CGM placement at post-intervention, as well as average total daily mealtime changes, may have influenced our findings.

Overall, this dissertation found that a relationship exists between PA with glucose concentrations and oxidative stress. However, glycemic variability may not be positively influenced by exercise training in non-diabetic overweight or obese adults even in the presence of improvements in biological markers of oxidative stress. Collectively, habitual PA may influence glycemic variability differently than structured PA, known as exercise, in our participants. However, these findings are speculative and further research is needed in this population.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%CV ..... Percent Coefficient of Variation

ANOVA ..... Analysis of Variance

A-TEAM ..... Aerobic Treadmill Exercise and Metabolism

AUC ..... Area Under the Curve

BMI ..... Body Mass Index

CERC ..... Clinical Exercise Research Center

CINAHL ..... Cumulative Index of Nursing and Allied Health Literature

CGM ..... Continuous Glucose Monitor

CONGA ..... Continuous Overlapping Net Glycemic Action

CONGA-n ..... Continuous Overlapping Net Glycemic Action of n Hours

CONGA-1 ..... Continuous Overlapping Net Glycemic Action of 1 Hour

CONGA-2 ..... Continuous Overlapping Net Glycemic Action of 2 Hours

CONGA-4 ..... Continuous Overlapping Net Glycemic Action of 4 Hours

CRD ..... Centre for Reviews and Dissemination

CRF ..... Cardiorespiratory Fitness

CVD ..... Cardiovascular Disease

ELISA ..... Enzyme-Linked Immunoabsorbant Assay

HbA1c ..... Hemoglobin A1c

HH:MM ..... Hour:Minute in 24-Hour System

HIIT ..... High-Intensity Interval Training

HR ..... Heart Rate

HRR ..... Heart Rate Reserve

KKW ..... Kilocalorie per Kilogram of Body Weight

LPA ..... Light-Intensity Physical Activity

LPA EE ..... Light-Intensity Physical Activity Energy Expenditure

MAGE ..... Mean Amplitude of Glucose Excursions

METs ..... Metabolic Equivalents

MODD ..... Mean of Daily Differences

MPA ..... Moderate-Intensity Physical Activity

MVPA ..... Moderate-to-Vigorous-Intensity Physical Activity

MVPA EE ..... Moderate-to-Vigorous-Intensity Physical Activity Energy Expenditure

OGTT ..... Oral Glucose Tolerance Test

PA ..... Physical Activity

PAEE ..... Physical Activity Energy Expenditure

SD ..... Standard Deviation

TDEE ..... Total Daily Energy Expenditure

$\dot{V}O_2$ ..... Volume of Oxygen Consumed

$\dot{V}O_{2max}$ ..... Maximal Volume of Oxygen Consumed

$\dot{V}O_{2peak}$ ..... Peak Volume of Oxygen Consumed

VPA ..... Vigorous-Intensity Physical Activity

WORDS ..... Weight Outlooks by Restriction of Diet and Sleep

CHAPTER 1

INTRODUCTION

Currently, ~70% of adults in the United States are considered overweight or obese with obesity largely considered the public health crisis of the current generation (Flegal MF, Kruszon-Moran D, Carroll MD, Fryar CD, & Ogden CL, 2016; Centers for Disease Control and Prevention 2017; National Institutes of Health 2018). The Centers for Disease Control and Prevention announced that the medical care cost of obesity and its associated disorders were $147 billion in 2008 (Finkelstein EA, Trogdon JG, Cohen JW, & Dietz, W, 2009; Centers for Disease Control and Prevention 2017). Being overweight or obese leads to an increased risk of development of diseases or diseased states such as impaired glucose tolerance and cardiovascular disease (Bray GA 2004; O'donovan G, Kearney EM, Nevill AM, Woolf-May K, & Bird SR, 2005). Moreover, fasting and postprandial hyperglycemia are independent risk factors for cardiovascular disease with and without the presence of type 2 diabetes mellitus and are increased in overweight and obesity (Mokdad AH, Bowman BA, & Ford ES, 2001; Hanire H, Bertrand M, Guerci B, Anduze Y, Guillaume E, & Ritz P, 2011; Little JP, Jung ME, Wright AE, Wright W, & Manders RJ, 2014). Unlike hepatic regulation of glucose in a fasted state, and how effective muscle glucose disposal is in a glucose challenged state, such as during an oral glucose tolerance test (OGTT), the measurement of glycemic variability allows for the observation of excursions that consider nadirs, peaks, and troughs of glucose concentrations rather than a single snapshot of fasting glucose and during an OGTT

Hermanides J, Vriesendorp TM, Bosman RJ, Zandstra DF, Hoekstra JB, & DeVries JH, 2010; Anderwald C, Gastaldelli A, Tura A, Krebs M, Promintzer-Schifferl M, Kautzy-Willer A, Stadler M, DeFronzo RA, Pacini G, & Bischof MG, 2011; Petersen MC, Vatner DF, & Shulman GI, 2017). Recently, increased glycemic variability has been shown to be associated with the risk of development of type 2 diabetes mellitus, with overweight and obese adults having higher glycemic variability compared to normal weight (Ma C-M, Yin F-Z, Wang R, Qin C-M, Lou D-H, & Lu Q, 2011). Additionally, it has been suggested that glycemic variability may be a more important clinical measurement of not only glucose metabolism, but cardiovascular and overall general health (Monnier L, Colette C, & Owens DR, 2008; Rodbard D 2011; Nusca A, Tuccinardi D, Albano M, Cavallaro C, Ricottini E, Manfrini S, Pozzilli P, & Di Sciascio G, 2018). As technology for the assessment of glycemic variability utilizing continuous glucose monitoring (CGM) continues to increase, the implications of this maker of metabolic health outside of a clinical setting becomes of further importance.

Furthermore, increased glycemic variability has recently been associated with oxidative stress and thus linked with increased risk of cardiovascular disease in people with and without type 2 diabetes mellitus (Saisho Y 2014). Oxidative stress plays a crucial role in the development of vascular complications leading to increased risk of development of cardiovascular disease. It is increased in overweight and obese populations, and further complicated by hyperglycemia (Pitocco D, Tesauro M, Alessandro R, Ghirlanda G, & Cardillo C, 2013). In 2006, Monnier et al., reported a strong correlation between glycemic variability assessed by mean amplitude of glucose excursions (MAGE) and continuous overlapping net glycemic action (CONGA), and

activation of oxidative stress in a type 1 and 2 diabetic population, which was the first study to utilize CGM to assess this relationship (Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, & Colette C, 2006). *In vitro* and *in vivo* animal studies indicate that glycemic variability, compared to sustained hyperglycemia, promotes oxidative stress and worsens vascular damage; however, how glycemic variability and oxidative stress relate to and interact with each other remains unclear (Saisho Y 2014). Glycemic variability has previously been shown to be associated with oxidative stress formation in type 2 diabetes mellitus, but no studies have been performed to examine the relationship between glycemic variability, specifically in a "free-living" condition, and oxidative stress in overweight or obese adults not diagnosed with diabetes (Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, & Colette C, 2006; Wentholt IME, Kulik W, Michels RP, Hoekstra JB, DeVries JH, 2008; Siegelaar SE, Barwari T, Kulik W, Hoekstra JB, & DeVries JH, 2011). Therefore, the potential relationship and clinical implications of the relationship between glycemic variability and oxidative stress, which serve as markers for the risk of development of metabolic and cardiovascular disease, needs to be further explored (Monnier L, Colette C, & Owens DR, 2008; Wentholt IME, Kulik W, Michels RPJ, Hoekstra JBL, & DeVries JH, 2008).

As of 2015, the Centers for Disease Control and Prevention estimated that ~20.5% of American adults met the minimum guidelines for being physically active (Centers for Disease Control and Prevention 2017). Decreased physical activity (PA) of light-intensity (LPA), moderate-intensity (MPA), vigorous-intensity (VPA), a combination of moderate-to-vigorous-intensity (MVPA), and even total PA are not only detrimental to fasting (Nygaard H, Grindaker E, Rønnestad BR, Holmboe-Ottesen G, &

Høstmark AT, 2017), but postprandial glucose concentrations (Høstmark AT, Ekeland GS, Beckstrøm AC, & Meen HD, 2006), and increase risk for the development of cardiovascular disease (Carnethon MR, Evans NS, Church TS, Lewis CE, Schreiner PJ Jacobs Jr DR, Sternfel B, & Sidney S, 2010) with no current knowledge surrounding whether sedentary time and PA of varying intensities are associated with glycemic variability or oxidative stress.

Additionally, evidence suggests regular exercise can influence PA and may provide a protective effect against exercise and non-exercise induced oxidative stress in healthy weight adults (Radak Z, Chung HY, & Got S, 2008); however, how this protective effect may be altered in persons with decreased glycemic control at risk for the development of diabetes remains uncertain (Sen CK, Atalay M, & Hänninen O, 1994; Atalay M, Laaksonen DE, Niskanen L, Uusitupa M, Hänninen O, & Sen SK, 1997). Moreover, there remains unclear evidence to suggest how exercise training may influence glycemic variability and oxidative stress in sedentary, overweight or obese, but otherwise healthy adults.

SCOPE OF THE STUDY

Therefore, the overall purpose of this dissertation is 1) to examine the current literature surrounding sedentary time and PA, glycemic variability, and oxidative stress, 2) to determine the associations between sedentary time and PA of varying intensities, glycemic variability, and oxidative stress in currently overweight or obese adults in a cross-sectional setting, and 3) to examine the effect a 12-week aerobic exercise intervention has on glycemic variability and oxidative stress in currently overweight or obese adults. Our central hypothesis is that the time spent being sedentary and performing

various intensities of PA will be associated with CGM assessed glycemic variability and oxidative stress, and that incorporation of an aerobic exercise intervention will decrease glycemic variability and improve measures of oxidative stress in overweight or obese adults.

Specific Aim 1

To perform a critical review of the current literature surrounding the relationship between sedentary time and PA, glycemic variability, and oxidative stress.

Sub-Aim 1.1: To summarize and describe the known relationship between sedentary time and PA, glycemic variability, oxidative stress.

Sub-Aim 1.2: To summarize how acute exercise affects glycemic variability.

Sub-Aim 1.3: To summarize how repeated bouts of exercise and exercise training affects glycemic variability.

Specific Aim 2

To examine the cross-sectional associations between sedentary time and PA, glycemic variability, and biological markers of oxidative stress in overweight or obese adults.

Sub-Aim 2.1: To examine the associations between daily sedentary time, glycemic variability, and oxidative stress.

Sub-Aim 2.2: To examine the associations between daily LPA and MVPA, glycemic variability, and oxidative stress.

Sub-Aim 2.3: To examine the associations between daily total PA, glycemic variability, and oxidative stress.

Specific Aim 3

To examine the effect of a 12-week aerobic exercise intervention on glycemic variability and oxidative stress.

Sub-Aim 3.1: To examine the effect of a 12-week aerobic exercise intervention on glycemic variability.

Sub-Aim 3.2: To examine the effect of a 12-week aerobic exercise intervention on oxidative stress.

CHAPTER 2

LITERATURE REVIEW

OVERVIEW

The prevalence of adults in the United States classified as either overweight or obese has continued to increase throughout the years, with ~39.8% or 93.9 million considered obese in 2015-2016 (Hales CM, Carroll MD, Fryar CD, & Ogden CL, 2017). Overweight and obesity remains a complex public health issue to address and results from a combination of causes and contributing factors, including, but not limited to behavioral factors, such as diet and physical activity, genetic and environmental factors, and even sociodemographic status (Centers for Disease Control and Prevention 2017). Adults classified as overweight or obese, when compared to healthy weight adults, are at an increased risk for all cause morbidity and mortality (National Institutes of Health 2013), and are at a greater predisposition for the development of type 2 diabetes mellitus, and cardiovascular disease risk factors and cardiovascular disease (National Institutes of Health 1998).

Glycemic variability has recently been thought of as a sensitive measurement for glycemic control, with excess glycemic variability shown to increase the risk for the development of type 2 diabetes mellitus in overweight or obese adults and may provide greater insight into metabolic health compared to fasting blood glucose concentration and during an OGTT (Umpierrez GE & Kovatchev BP, 2018). Glycemic variability allows for the inclusion of glucose excursions when measuring glycemic control and allows for a

more sensitive analysis compared to other clinical measures of glucose metabolism and tolerance. As technology has continued to advance, glycemic variability assessment has become a non-invasive procedure utilizing CGM technology, which allows for the device be worn during everyday life and aids in the management of not only diabetes, but assessment for those at risk for the development of type 2 diabetes mellitus (National Institutes of Health 2017). Thus, glycemic variability measurement utilizing CGM technology plays an important role in assessment of diabetes risk in overweight or obese adults by allowing for observation in a "free living" environment in addition to common clinical measures glycemic health.

Oxidative stress has been utilized as a clinical measurement for the development of atherosclerosis, which is the leading cause of cardiovascular disease, and has been associated with the development and progression of cardiovascular disease risk factors, such as overweight and obesity, hypertension, and dyslipidemia, and cardiovascular disease (Gracia KC, Llanas-Cornejo D, & Husi H, 2017). Additionally, overweight and obesity has been recently thought to increase chronic low grade systemic inflammation and, in turn, induce greater amounts of oxidative stress leading to further overweight and obesity related complications (Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, & Chen H, 2003). Therefore, oxidative stress measurement serves as an excellent biological marker for risk of the development of cardiovascular disease risk factors and cardiovascular disease, especially in an overweight or obese population. Additionally, increased glycemic variability has been shown to be associated with greater reactive oxygen species production, oxidative stress, and vascular damage, compared to chronic hyperglycemia notably found in type 2

diabetes (Saisho Y 2014). Hence, management of glycemic variability may reduce oxidative stress and the risk for the development of cardiovascular disease in those at risk for the development of type 2 diabetes mellitus.

According to the Behavioral Risk Factor Surveillance System, in 2017 greater than 25% of adults in the United States engage in no leisure time PA with less than 50% meeting the minimum physical activity guidelines (Centers for Disease Control and Prevention 2017). Increased sedentary time and decreased PA of any intensity, including leisure time PA, are known to affect overall health and has been shown to increase the risk for the development of type 2 diabetes mellitus, and cardiovascular disease risk factors and cardiovascular disease (Flegal KM, Carroll MD, & Ogden CL, 2002; Carter S, Hartman Y, Holder S, Thijssen DH, & Hopkins ND, 2017; Joseph JJ, Echouffo-Tcheugui JB, Golden SH, Chen H, Jenny NS, Carnethon MR, Jacobs Jr D, Burke GL, Vaidya D, Ouyang P, & Bertoni AG, 2017). Time spent sedentary and performing PA have been widely studied in regards to their positive effects on overweight or obesity, glucose tolerance, and cardiovascular health (Jiménez-Pavón D, Ortega FB, Valtueña J, Castro-Piñero J, Gómez-Martínez S, Zaccaria M, Gottrand F, Molnár D, Sjöström M, González-Gross M, Castillo MJ, Moreno LA, & Ruiz JR, 2012; Miguel-Berges ML, Reilly JJ, Moreno Aznar LA, & Jiménez-Pavón D, 2018). However, the relationship between metabolic and cardiovascular health and PA remains complex with the majority of data suggesting that PA of any intensity may promote overall health benefits (Ortega FB, Artero EG, Jiménez-Pavón D, & Ruiz JR, 2018). Therefore, further examination as to how sedentary time and PA affect glycemic variability and oxidative stress in overweight or obese adults is required.

Exercise has often been utilized as a part of the therapeutic method to combat overweight and obesity, diabetes risk, and cardiovascular disease. Acute and chronic exercise have both been shown to improve glycemic control and glucose tolerance (Goodyear LJ & Kahn BB, 1998), but no studies to date have examined the effect of chronic aerobic exercise on glycemic variability. Additionally, even though acute exercise is shown to increase oxidative stress post-exercise, physiological adaptations to chronic aerobic exercise has been shown to decrease oxidative stress at rest and post-exercise (Reid MB 1985; Baltaci SB, Mogulkoc R, & Baltaci AK, 2016). Moreover, no studies have sought to examine how glycemic variability and oxidative stress change in overweight or obese adults undergoing chronic aerobic exercise. Thus, this dissertation proposes to fill some of these gaps by direct assessment of changes in clinically relevant indices of glycemic variability and measurement of biomarkers associated with oxidative stress in overweight or obese adults undergoing a 12-week aerobic exercise intervention.

Overweight, Obesity, and Cardiometabolic Risk

The prevalence of overweight and obesity has continued to increase over the past several decades, is considered an important public health issue, and has been identified as 1 of the 10 leading health indicators (Office of the Surgeon General 2001; Hedley AA, Ogden CL, & Johnson CL, 2004; Fryar CD, Carroll MD, & Ogden CL, 2016; Ogden C, Carroll MD, Lawman HG, Fryar CD, Kruszon-Moran D, Kit BK, & Flegal KM, 2016). The state of being overweight or obese leads to an increase risk of a number of medical conditions and substantial health care costs (Birmingham CL, Muller JL, Palepu A, Spinelli JJ, & Anis AH, 1999; Katzmarzyk PT & Janssen I, 2004; McCormick B & Stone I, 2007; Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, & Anis AH, 2008).

This includes, but is not limited to, type 2 diabetes mellitus (Hartemink N, Boshuizen HC, Nagelkerke NJ, Jacobs MA, & van Houwelingen HC, 2006) and cardiovascular disease (Wilson PWF, D'Agostino RB, Sullivan L, Parise H, & Kannel WB, 2002).

According to the American Diabetes Associations and the Centers for Disease Control and Prevention, 30.3 million adults in the United States have diabetes, while an additional 84.1 million have prediabetes (National Center for Chronic Disease and Prevention and Health Promotion 2017). Overweight and obesity have been associated with the development of type 2 diabetes mellitus, which leads to a further increase in all cause morbidity and mortality for adults in the United States (Mokdad AH, Bowman BA, Ford ES, Vivicor F, Marks JS, & Koplan JP, 2001). Adults who are obese have 7.37 greater odds of diagnoses of type 2 diabetes mellitus with the diagnosis of type 2 diabetes mellitus increasing by ~8.2% in recent years (Mokdad AH, Ford ES, & Bowman BA, 2003). Additionally, ~90% of type 2 diabetics have a body mass index (BMI) of ≥23 kg/m^2, which, even though this includes adults considered healthy weight, remains one the most significant contributors to decreased overall health (Kopelman P 2007; Haslam DW & James WPT, 2005). Furthermore, the increase in relative risk for the development of type 2 diabetes mellitus has been reported as 1.18 per unit increase of BMI (Hartemink N, Boshuizen HC, Nagelkerke NJD, Jacobs MAM, & van Houwelingen HC, 2006). However, the relationship between BMI and the relative risk for type 2 diabetes mellitus and several studies suggest there may be a threshold effect and certain cut-point of BMI once considered overweight (Modan M, Karasik A, Halkin H, Fuchs Z, Lusky A, Shitrit A, & Modan B, 1986).

Cardiovascular disease is the primary cause of mortality in the United States (Kochanek KD, Murphy SL, Xu J, & Tejada-Vera B, 2016) and about half of adults in the United States have been diagnosed with cardiovascular disease risk factors, such as hypertension or dyslipidemia (Fryar CD, Chen T, & Li X, 2012). The state of being overweight or obese has been established as a major risk factor for the development of cardiovascular disease, specifically if excess body fat has been primarily stored centrally or abdominally (Van Gaal LF, Mertens IL, & De Bock CE, 2006; Din-Dzietham R, Liu Y, Bielo MV, & Shamsa F, 2007), and has been associated with hypertension, dyslipidemia, and elevated levels of C-reactive protein, all of which increase the risk for development of cardiovascular disease (Ritchie SA & Connell JM, 2007). Additionally, overweight and obese men with cardiovascular disease risk factors and cardiovascular disease have a higher risk for all cause and cardiovascular disease mortality compared with healthy weight men (Wei M, Kampert, JB, Barlow CE, Nichaman MZ, Gibbon LW, Paffenbarger Jr RS, & Blair SN, 1999). Furthermore, it has been hypothesized that being overweight and obese does not directly translate to increased risk of cardiovascular disease; however, it may be the interaction of obesity developed insulin resistance and glucose intolerance related cardiovascular disease risk factors that leads to the development of cardiovascular disease (Reaven G 2005). Therefore, the development of type 2 diabetes mellitus may play a contributing factor in the development of cardiovascular disease risk factors and cardiovascular disease in overweight or obese adults.

The Framingham Study was among the initial observational longitudinal studies to examine diabetes and cardiovascular disease, which found a significant increase in

cardiovascular disease risk factors and mortality in men and women diagnosed with type 2 diabetes mellitus compared to their non-diabetic counterparts (Kannel WB & McGee DL, 1979). This study pioneered research related to the risk for development of diabetes and cardiovascular disease. The Multiple Risk Factor Intervention Trial reported that cardiovascular disease mortality was much higher for diabetic versus nondiabetic men for every age, race, and income (Stamler J, Vaccaro O, Neaton JD, Wentworth D, & The Multiple Risk Factor Intervention Trial Research Group, 1993). Furthermore, risk for cardiovascular disease mortality increased more steeply for diabetic men for each diagnosis of each additional cardiovascular disease risk factor (Stamler J, Vaccaro O, Neaton JD, Wentworth D, & The Multiple Risk Factor Intervention Trial Research Group, 1993). Additionally, hyperglycemia, which normally accompanies type 2 diabetes mellitus, has major direct and indirect effects on the human vascular tree, which are considered the major sources of morbidity and mortality in type 2 diabetes mellitus, with the negative effects of hyperglycemia including macrovascular complications such as coronary artery disease (Fowler MJ 2008).

GLYCEMIC VARIABILITY

Chronic hyperglycemia is a primary risk factor for the development of complications in type 2 diabetes mellitus; however, it is believed that frequent or exacerbated fluctuations in glucose concentrations may independently contribute to diabetes-related complications (Suh S & Kim JH, 2015). Glycemic variability refers to the peaks, troughs, and nadirs in glucose concentration levels and alludes to blood glucose oscillations that occur throughout the day, which includes glycemic excursions during periods of hypoglycemia and hyperglycemia. Glycemic variability is not always a

negative consequence of physiological dysfunction, rather sometimes it is a product of circadian rhythm of endogenous hormones, ingestion of calorie containing items, or PA, and is observed in individuals with normal glucose tolerance (Wang S, Lv L, Yang Y, Chen D, Liu G, Chen L, Song Y, He L, Li X, Tian H, Jia W, & Ran X, 2012). However, glycemic variability has been observed to be increased in sedentary, overweight or obese individuals (Salkind SJ, Huizenga R, Fonda SJ, Walker MS, & Vigersky RA, 2014), who have a greater risk for the development of impaired glucose regulation and cardiovascular disease morbidity and mortality (Nalysnyk L, Hernandez-Medina M, & Krishnarajah G, 2010; Gerbaud E, Darier R, Montaudon M, Beauvieux M-C, Coffin-Boutreux C, Coste P, Douard H, Ouattara A, & Catargi B, 2019). In order to effectively determine glycemic control in overweight and obese adults at risk for the development of type 2 diabetes mellitus or cardiovascular disease, glycemic variability must be assessed.

Intra- versus inter-day variability refers to the outcomes of data either collected on the same day or on different days, which is of importance when considering glycemic variability, as variability throughout the day may be more indicative of glucose metabolism as opposed to over multiple days, or even vice-versa. Even though intra-day glycemic variability can be useful throughout a 24-hour period, more sensitive measures are preferred to account for the degree of glucose excursions that occur over more than a single day. Measuring inter-day glycemic variability in addition to intra-day glycemic variability allows insight into the differences between day-to-day glycemic profile (Mori H, Okada Y, Kurozumi A, Narisawa M, & Tanaka Y, 2017). Glycemic variability may not only be dependent on each individual's lifestyle but may change day-to-day depending on their changes in daily lifestyle factors such as diet or PA patterns

throughout the week (Bennet B & Sothern MS, 2009). With this in mind, measuring intra- and inter-days glycemic variability provides a greater insight into glycemic health and how glycemic variability may be influenced by changes in an individual's daily life. However, most studies have not examined the intra- and inter-day glycemic variability during either the same observation period or following a lifestyle intervention. Therefore, when examining glycemic variability in overweight or obese adults at risk for the development of impaired glucose tolerance and type 2 diabetes mellitus, it is pertinent to investigate both the intra- and inter-day glycemic variability that will allow for a more in-depth insight into glycemic control.

The MAGE was introduced as a measurement of glycemic variability to aid in the treatment and further refinement of the characterization of diabetic instability, and has been shown to be lower in non-diabetic subjects compared to subjects with stable and unstable type 2 diabetes mellitus (Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, & Taylor WF, 1970). MAGE has traditionally been estimated with a graphical approach that represents the arithmetic mean of the difference between consecutive peaks and troughs exceeding one standard deviation around the respective 24-hour glucose mean (Service FJ & Nelson RL, 1980). Assessment of MAGE over multiple days allows for an impactful outlook into inter-day glycemic variability and is considered an essential index for glycemic variability assessment (Monnier L, Colette C, & Owens DR, 2008; Fritzsche G, Kohnert K-D, Heinke P, Vogt L, & Salzsierder E 2011). In additional to MAGE, CONGA is thought of an important additional measure of glycemic variability as CONGA can be analyzed in shorter time components throughout the day from a set 1, 2, 3, or 4 hours at a time with decreased validity for utilization after

4 hours (Monsod TP, Flanagan DE, Rife F, Saenz R, Caprio S, Sherwin RS, & Tamborlane WV, 2002). The CONGA equation was originally derived by McDonnell, et al., to allow for the time periods corresponding to different activities performed throughout the day (CONGA of 1 hour; CONGA-1), time consuming snacks throughout the day (CONGA of 2 hour; CONGA-2), or time between standard meals, such as breakfast, lunch, and dinner (CONGA of 4 hour; CONGA-4) (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005; Rodrigues R, de Medeiros LA, Cunha LM, Garrote-Filho MDS, Bernardino Neto M, Jorge PT, Resende ES, & Penha-Silva N, 2018). This calculation of CONGA allows for each glucose concentration observation after the initial observation to be identified and the difference between the initial observation and the second observation is calculated and is considered the standard deviation of the differences with higher values indicating increase glycemic variability (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005). Utilizing both MAGE and CONGA measurements of glycemic variability allow for a broad-spectrum approach to establish intra- and inter- day glycemic variability. This method also allows for a validated inter-day measurement of glycemic variability, while also incorporating a sensitive measure of intra-day glycemic variability. Measuring both intra- and inter-day glycemic variability may also serve as a tool for the examination of time spent hyperglycemic and hypoglycemic as both are considered to be increased risks for the development of type 2 diabetes mellitus (Satya Krishna SV, Kota SK, & Modi KD, 2013). In turn, this method permits a greater insight into glycemic variability as not only an all-encompassing clinical measure, but practical measure of glycemic control and metabolic health.

As previously stated, the state of being overweight or obese leads to increased risk for the development of type 2 diabetes mellitus (Mokdad AH, Bowman BA, Ford ES, Vivicor F, Marks JS, & Koplan JP, 2001). However, overweight and obesity also leads to impaired glucose tolerance prior to diagnosis of type 2 diabetes mellitus (Ferrannini E & Camastra S, 1998). The major limitation of evaluating glucose tolerance, especially in overweight or obese adults, is a result of only testing fasting blood glucose levels, or during a glucose challenged state, such as an OGTT, which provides a snapshot of glycemic health as opposed to observation in a "free-living" environment. Glycemic variability has been reported to be higher in adults with type 2 diabetes mellitus compared to adults with normal glucose tolerance (Mazze RS, Strock E, Wesley D, Borgman S, Morgan B, Bergenstal R, & Cuddihy R, 2008). Previously, Ma et al., found that glycemic variability was higher in abdominally obese compared to non-abdominally obese men (Ma C-M, Yin F-Z, Wang R, Qin C-M, Liu B, Lou D-H, & Lu Q, 2011). Additionally, glycemic variability has been shown to be higher in obese adults who have normal glucose tolerance and insignificantly different from prediabetic adults (Salkind SJ, Huizenga R, Fonda SJ, Walker MS, & Vigersky RA, 2014). Thus, glycemic variability may provide greater insight and be more clinically relevant to evaluate metabolic health.

Oxidative Stress

The imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to vascular damage has primarily been utilized as the definition of oxidative stress (Keaney Jr JF 2000). Additionally, oxidative stress has been coined a phenomenon caused by an imbalance between production and accumulation of reactive

oxygen species in the cells of tissues, as well as the ability of a biological system to detoxify these reactive products (Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, & Bitto A, 2017). When reactive oxygen species production increases, creating greater oxidative stress, they increase the risk of harmful effects on important cellular structures such as proteins, lipids, and nucleic acids (Wu JQ, Kosten TR, & Zhang XY, 2013). Furthermore, evidence suggests that oxidative stress may be responsible, with varying degree of importance, for the onset and/or progression of several diseases, such as type 2 diabetes mellitus and cardiovascular disease (Taniyama Y & Griendling KK, 2003). Reactive oxygen species, when maintained at low or moderate levels, are of crucial importance to not only metabolic and cardiovascular, but overall health (Pacher P, Beckman JS, & Liaudet L, 2007). Moreover, oxidative stress plays a key role in the risk for the development of type 2 diabetes mellitus, cardiovascular disease risk factors and cardiovascular disease, as well as all-cause mortality in overweight or obese adults (Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, & Arrigo T, 2015).

Nitric oxide is an inorganic free radical gas that is synthesized by vascular endothelial cells from L-arginine via nitric oxide synthases as a transcellular signal of oxidative stress (Knowles RG & Moncada S, 1994). Nitric oxide is considered a potent vasodilator released by vascular endothelial cells and accounts for the relaxation of vascular tissue, inhibits platelet aggregation and platelet adhesion attributed to endothelium-derived relaxing factor, and reduces proliferation of the intima of vascular tissue (Palmer RMJ, Ashton DS, & Moncada S, 1998). Enhanced nitric oxide inhibition or reduced synthesis of nitric oxide is often associated with cardiovascular disease risk

factors (Förstermann U, Nakane M, Tracey WR, & Pollock JS, 1993). Additionally, reactive oxygen species, that contribute to the underlying mechanisms of vascular dysfunction and oxidative stress, may reduce the amount of bioactive nitric oxide by chemical inactivation of nitric oxide synthase, which may in-turn cause the nitric oxide synthase to become dysfunctional and contribute further to oxidative stress (Förstermann U 2010). Increased bio-active concentrations of nitric oxide are often associated with a decrease in reactive oxidative species, a subsequent decreased oxidative stress, and, in-turn, risk for the development of cardiovascular disease risk factors (Förstermann U & Münzel T, 2006). Inversely, myeloperoxidase, a heme protein released by leukocytes, plays a crucial role in inflammation and oxidative stress at the cellular level, which affects the arterial endothelium and impairment of nitric oxide-induced vascular relaxation, and has been speculated to be a major oxidative stress pathway (Anatoliotakis A, Deftereos S, Bouras G, Giannopoulos G, Tsounis D, Angelidis C, Kaoukis A, & Stafanadis C, 2013). Similarly, to nitric oxide, there exists mechanistic links between myeloperoxidase activity and manifestations of cardiovascular disease (Brennan M-L & Hazen SL, 2003). Therefore, evidence suggests determining both nitric oxide and myeloperoxidase together when examining oxidative stress as nitric oxide may inhibit production of myeloperoxidase, while myeloperoxidase may decrease nitric oxide concentrations.

It has been well established that obesity is associated with low-grade chronic systemic inflammation in adipose tissue, which is a condition influenced by the activation of adipose tissue immune response that promotes oxidative stress and is associated with type 2 diabetes mellitus and cardiovascular disease (Alberti K & Zimmet PZ, 1998).

Susceptibility to oxidative damage during times of increased oxidative stress is even greater in obesity due to depleted antioxidant sources, which are used to aid in clearance of reactive oxygen species associated with oxidative stress, compared to healthy weight (Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, & Chamari M, 2007). There are several prevailing theories as to the mechanism involved in the generation of oxidative stress in obesity, including the release of adipokines (adipose tissue inflammatory molecules), increased cellular apoptosis (cellular death), and excessive hepatic lipogenesis (formation of adipose tissue) (Hensley K, Robinson KA, Gabbita SP, Salsman S, & Floyd RA, 2000). Biomarkers of oxidative damage are higher in individuals with obesity and correlate directly with BMI and the percentage of body fat (Pihl E, Zilmer K, Kullisaar T, Kairane C, Mägi A, & Zilmer M, 2006). However, the evidence remains inconsistent as an inverse relationship between body fat, central adiposity, and antioxidant capacity has been suggested (Chrysohoou C, Panagiotakos DB, Pitsavos C, Skoumas I, Papademetriou L, Economou M, & Stefanadis C, 2007). Insulin resistance is a characteristic feature of type 2 diabetes mellitus and obesity and promotes oxidative stress-induced atherogenesis even in the absence of hyperglycemia (Yip J, Facchini FS, & Reaven GM, 1998). Likewise, Du et al., provided evidence that insulin resistance increases mitochondrial reactive oxygen species production implicated in hyperglycemia-induced vascular damage and inactivation of enzymes involved in atherogenesis leading to the development of atherosclerosis related to obesity and type 2 diabetes mellitus (Du X, Edelstein D, Obici S, Higham N, Zou MH, & Brownlee M, 2006; Hulsmans M, Van Dooren E, & Holvoet P, 2012).

Oxidative stress has been previously shown to be associated with glycemic variability in type 1 and type 2 diabetes mellitus (Monnier L, Mas E, & Ginet C, 2006; Rodrigues R, de Medeiros LA, Cunha LM, Garrote-Filho MDS, Bernardino Neto M, Jorge PT, Resende ES, & Penha-Silva N, 2018). Additionally, a study performed by Rizzo et al., in patients with type 2 diabetes mellitus inadequately controlled by metformin found that MAGE correlated with biological markers of oxidative stress (Rizzo MR, Barbieri M, Marfella R, & Paolisso G, 2012) However, no studies have sought to examine the relationship in overweight or obese adults, who are at higher risk for developing cardiometabolic disorders. Hence, when examining the effect of overweight and obesity-induced oxidative stress, it remains important to not only assess oxidative stress, but risk factors for the development of type 2 diabetes mellitus, such as increased glycemic variability, in overweight and obese adults.

Glycemic Variability and Oxidative Stress

Management of glycemic variability may reduce oxidative stress-induced cardiovascular disease in patients with diabetes (Saisho Y 2014). Ceriello et al., evaluated the effect of oscillating glucose concentrations on endothelial function and oxidative stress in normoglycemic and adults diagnosed with type 2 diabetes mellitus and found that that higher oscillating glucose concentration, increased glycemic variability, results in greater endothelial dysfunction and oxidative stress than lower oscillating glucose concentration, decreased glycemic variability, in both normoglycemia and type 2 diabetes mellitus (Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, Boemi M, & Giugliano D, 2008). Di Flaviani et al., noted a significant association between intra-day glycemic variability and oxidative stress in adults with type 2 diabetes mellitus with and

without metformin administration (Di Flaviani A, Picconi F, Di Stefano P, Giordani I, Malandrucco I, Maggio P, Palazzo P, Sgreccia F, Peraldo C, Farina F, Frajese G, & Frontoni S, 2011). Additionally, Monnier et al., reported a strong positive correlation between inter-day glycemic variability and oxidative stress in adults diagnosed with type 2 diabetes mellitus (Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, & Colette C, 2006). However, most studies performed to evaluate the relationship between glycemic variability and oxidative stress have been in diabetic, not overweight or obese, normoglycemic adults at risk for the development of type 2 diabetes mellitus. Thus, this creates a gap in the literature that must be addressed when assessing not only if a relationship exists between glycemic variability and oxidative stress, but to establish to what extent this relationship exists in overweight or obese adults.

Relationship between Sedentary Time, Physical Activity, Glycemic Variability, and Oxidative Stress

It has been well established that decreasing sedentary time and increasing PA of all intensities is beneficial to overall health and may be potentially altered in overweight or obese adults. Additionally, overweight and obesity increases the risk for the development of type 2 diabetes mellitus and cardiovascular disease, which is further exacerbated by physical inactivity (Chan CB, Ryan DAJ, & Tudor-Locke CT, 2004; Van Gaal FL & Maggioni AP, 2013). However, there is little information regarding the relationship between sedentary time and physical activity, glycemic variability, and oxidative stress. Increase sedentary time and decreased PA of any intensity has been shown to be detrimental to overall health; however, how glycemic variability and oxidative stress outcomes may be associated with sedentary behavior and PA in overweight or obese adults remains relatively unknown. It has been reported that active

men and women appear to be protected against the hazards of overweight or obesity, including a stronger protective effect in obese adults compared to their healthy weight or overweight counterparts when examining diagnosis of type 2 diabetes mellitus, cardiovascular disease risk factors and cardiovascular disease mortality (Blair SN & Brodney S, 1999). However, it has been observed that overweight or obese adults tend to spend more time sedentary and perform less PA than healthy weight adults (Tudor-Locke C, Brashear MM, Johnson WD, & Katzmarzyk PT, 2010). Cooper et al., found that healthy weight and overweight adults performed similar amounts of PA, while obese adults performed less PA than healthy weight or overweight adults (Cooper AR, Page A, Fox KR, & Mission J, 2000). Even though decreasing sedentary time and increasing PA is believed to benefit metabolic and cardiovascular health, the results remain controversial regarding the effect on overweight and obese adults, as the energy cost of PA is not believed to be a major cause of obesity, rather a primary contributor and remains a public health strategy of choice in the prevention of obesity (Hill JO & Melanson EL, 1999).

Sedentary Time, Physical Activity, and Glycemic Variability

Limited data exist regarding the effect of sedentary time and PA on glycemic variability; however, it is known that decreasing time spent sedentary and increasing PA improves fasting blood glucose and glucose control during an OGTT or timed feeding in normoglycemic and diabetic populations (Dunstan DW, Barr EL, Healy GN, Salmon J, Shaw JE, Balkau B, & Owen N, 2010; Veerman JL, Healy GN, Cobiac LJ, Vos T, Winkler EA, Owen N, & Dunstan DW, 2012). In nondiabetic adults, sedentary behavior and decreased PA have been independently associated with increased risk for abnormal

glucose metabolism, elevated fasting insulin concentration, and impaired glucose tolerance (Helmerhorst HJ, Wijndaele K, Brage S, Wareham NJ, & Ekelund U, 2009; Ford ES, Zhao G, & Li C, 2010; Thorp AA, Healy GN, Owen N, Slamon J, Ball K, Shaw JE, & Dunstan DW, 2010; Lahjibi E, Heude B, Dekker JM, Højlund K, Laville M, Nolan Jn & Balkau B, 2013). Furthermore, in adults diagnosed with type 2 diabetes mellitus, more time spent sedentary was predictive of significant increases in time spent in hyperglycemia (Fritchi C, Park, H, Richardson, A, Park C, Collins EG, Mermelstein R, Riesche L, & Quinn L, 2016). Swindell et al., evaluated the impact between accelerometer-based measurement of sedentary time and PA of varying intensities, and cardiometabolic risk in adults with prediabetes, and found that MVPA and total PA counts was negatively associated with 2-hour glucose concentration (Swindell N, Mackintosh K, McNarry M, Stephens JW, Sluik D, Fogelholm M, Drummen M, MacDonald I, Martinez JA, Handjieva-Darlenska T, Poppitt SD, Brand-Miller J, Larsen TM, Raben A, & Stratton G, 2018). Even though these studies highlighted that sedentary time and time spent performing PA are shown to be related to glucose concentration and glucose tolerance, very few studies have emphasized this relationship with glycemic variability. Paing et al., sought to examine the associations between objectively measured sedentary time and breaks in sedentary time with 24-hour glycemic control in type 2 diabetes mellitus and found that increased sedentary time was found to be positively associated with time spent hyperglycemic, with more breaks in sedentary time positively associated with time spent euglycemic (Paing AC, McMillan KA, Kirk AF, Collier A, Hewitt A, & Chastin SFM, 2018). The prevailing theory for the mechanism of action is that adults, even normoglycemic, overweight or obese adults, that engage in greater

amounts of PA have increased insulin-stimulated glucose control and skeletal muscle glucose disposal than those who spend more time performing sedentary behavior (Berger M, Kemmer FW, Becker K, Herberg L, Schwenen M, Gjinavci A, & Berchtold, 1979; Hollenbeck CB, Haskell W, Rosenthal M, & Reaven GM, 1985). However, the relationship between sedentary time and PA of any intensity has only been minimally and inadequately evaluated in those who have developed impaired glucose metabolism and has yet to be examined in a population at risk for the development of type 2 diabetes mellitus, such as sedentary, overweight or obese adults.

Sedentary Time, Physical Activity, and Oxidative Stress

As limited as the data is on the relationship between sedentary time and PA with glycemic variability, there are even more limited findings available on the relationship between sedentary time and PA with oxidative stress. Sedentary lifestyle is a major risk factor for cardiovascular disease and is primarily believed to be due to oxidative stress induced arterial stiffness (Lessiana G, Santilli F, Boccatonda A, Iodice P, Liana R, Tripaldi R, Saggini R, & Davi G, 2016). Additionally, those who engage in PA has been shown to have lower oxidative stress compared to their sedentary counterparts (Carraro E, Schilirò T, Biorci F, Romanazzi V, Degan R, Buonocore D, Verri M, Dossena M, Bonetta S, & Gilli G, 2018). Furthermore, Yang et al., examined the association between PA and oxidative stress in a large sample of generally healthy women and found that higher levels of PA were associated with a decrease in oxidative stress, with an even greater association in a subset of women who performed vigorous-intensity PA (Yang S, Jensen MK, Mallick P, Rimm EB, Willett WC, & Wu T, 2015). However, this is one of the only studies which directly evaluated how sedentary time and PA of varying

intensities related to oxidative stress, but only utilized a subjective PA questionnaire to derive PA intensities, as opposed to objective accelerometry-derived measures of PA. Therefore, there remains a need to examine how objectively measured sedentary time and PA relate to oxidative stress, especially in overweight and obese adults at an increased risk for development of cardiovascular disease.

Impact of Aerobic Exercise on Glycemic Variability and Oxidative Stress

Aerobic Exercise and Glycemic Variability

Exercise is defined as structured PA performed with an underlying purpose or goal, and has been shown to influence insulin resistance and glucose tolerance, as well as is commonly utilized as a treatment for both non-insulin and insulin-dependent type 2 diabetes mellitus (Joslin EP, Root HF, White P, & Marble A, 1935; Goodyear LJ & Kahn BB, 1998). When discussing glucose homeostasis, even a single bout of exercise can positively impact whole-body glucose disposal and increase the uptake of glucose into skeletal muscle (Pruett EDR & Oseid S, 1970; Bogardus C, Thuillex P, Ravussin E, Vasquez B, Narimiga M, & Azhar S, 1983; Richter EA, Mikines KJ, Galbo H, & Kiens B, 1989; Wang X, Patterson BW, Smith GI, Kampelman J, Reeds DN, Sullivan SA, & Mittendorfer B, 2013), which may persist several hours after completion of exercise (Devlin JT & Horton ES, 1985; Devlin JT, Hirshman MF, Horton ES, & Horton ED, 1987; Mikines KJ, Sonne B, Farrell PA, Tronier B, & Galbo H, 1988). Another study even sought the impact of frequent breaks from sitting with and without moderate exercise and found that 24-hour glycemic control was positively impacted, with a more positive impact in glycemic control found in those undergoing additive exercise, suggesting that exercise may improve glucose homeostasis in addition to decreasing time

spent sedentary (Blankenship JM, Granados K, & Braun B, 2014). Furthermore, epidemiological studies have determined that chronic exercise can reduce the risk for developing non-insulin-dependent diabetes (Helmrich SP, Ragland DR, Leung RW, & Paffenbarger RS, 1991; Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, Rosner B, Hennekens CH, & Speizer FE, 1991; Manson JE, Nathan DM, Krolewski AS, Stampfer MJ, Willett WC, & Hennekens CH, 1992). A brief review and preliminary evidence published by Holloszy et al., suggest that a decline in glucose tolerance and insulin sensitivity can be prevented by performing regular exercise, and that prolonged and frequent exercise can normalize glucose tolerance by decreasing resistance to insulin in some individuals with impaired glucose tolerance (Holloszy JO, Schultz J, Kusnierkiewicz J, Hagberg JM, & Ehsani AA, 1986). However, no studies have evaluated glycemic variability following a chronic exercise intervention. Little et al., examined the impact of acute high-intensity interval training compared to continuous moderate-intensity exercise on postprandial hyperglycemia following standardized dietary conditions over 24 hours in overweight or obese adults, and found that overall postprandial glucose response and area under the curve reduced in both exercise groups compared to matched control group (Little JP, Jung ME, Wright AE, Wright W, & Manders RJF, 2014). An additional study by Figueira et al., evaluated the effects of a single session of aerobic exercise alone or aerobic exercise combined with resistance exercise on glucose levels and glycemic variability in type 2 diabetes mellitus currently taking metformin, resulted in no significant differences between either exercise condition in glucose concentration or glycemic variability, and decreased glucose concentrations and glycemic variability following both exercise conditions when conventional analysis

of glycemic variability (e.g. MAGE) was performed (Figueira FR, Umpierre D, Casali KR, Tetelbom PS, Henn NT, Ribeiro JP, & Schaan BD, 2013). Therefore, these previously mentioned studies necessitate the further exploration as to how glycemic variability may be influenced by structured exercise following a chronic exercise intervention. As well as, the less than ideal literature available regarding alterations in glycemic variability in adults that are sedentary, overweight or obese and at an increased risk for the development of type 2 diabetes mellitus undergoing an aerobic exercise intervention remains to be addressed.

Aerobic Exercise and Oxidative Stress

Cardiovascular disease is a largely preventable condition with many of the cardiovascular risk factors considered modifiable by leisure time PA, which includes exercise (Warburton DE, Nicol CW, & Bredin SS, 2006; Hamer M & Chida Y, 2008; Agarwal S 2012). Exercise has been suggested to induce oxidative stress acutely, which may subside following chronic physiological adaption to exercise and potentially be considered an antioxidant (Bouzid MA, Filaire E, Matran R, Robin S, & Fabre C, 2018). Acutely, accumulating evidence suggests that reactive oxygen species and, in turn, oxidative stress is generated during exercise and modulate muscle contraction, which contributes to the development of muscle fatigue (Reid MB 1985). However, oxidative stress in the context of chronic exercise and cardiovascular health has a differing relationship. Chronic, moderate aerobic exercise has been demonstrated to be useful in the prevention of oxidative stress, and, in turn serves as primary and secondary protection from oxidative stress-induced cardiovascular disease (Baltaci SB, Mogulkoc R, & Baltaci AK, 2016). Furthermore, it is believed and has been reported that adults who engage in

regular exercise, due to an adaptive physiological response, accumulate lower levels of reactive oxygen species and oxidative stress at rest and following a single bout of exercise compared to untrained adults (Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A, & Neri LM, 2018). Miyazaki et al., evaluated whether high-intensity endurance training would alleviate exercise-induced oxidative stress in untrained males following 12-weeks during which all participants exercised for 60 minutes a day, 5 days per week at 80% maximal exercise heart rate, and found an increase in antioxidant enzyme activity and subsequent decrease in oxidative stress following exercise training at rest and following an exhaustive exercise bout (Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Toshinai K, Ha S, Ji LL, & Ohno H, 2001). However, the intensity of the exercise in this study was high and may not be generalizable to those who meet the minimum guideline for participating in exercise. Medeiros et al., aimed to evaluate oxidative stress in obese adults undergoing 5-8 weeks of moderate-intensity concurrent training under two differing conditions, 3 days per week compared to 5 days per week for 70 minutes each session, and found that measures of oxidative stress decreased in both concurrent training groups, with the group completing 3 days per week eliciting a greater decrease in measures of oxidative stress (Medeiros NS, de Abreu FG, Colato AS, de Lemos LS, Ramis, TR, Dorneles GP, Funchal C, & Dani C, 2015).

Additionally, potential changes in glycemic variability due to exercise training may contribute to this observed reduction in oxidative stress after exercise training, which has yet to be elucidated. Thus, when examining oxidative stress, it is important to examine how it changes along with glycemic variability following aerobic exercise

training, which further implores the inclusion of glycemic variability as an important marker or overall health due to its potential relationship with oxidative stress.

CHAPTER 3

GENERAL METHODOLOGY

The first study that comprises this dissertation is a critical review of sedentary time and PA, glycemic variability assessed via CGM technology, and measures of oxidative stress in overweight or obese individuals. The second study combines two clinical trials, Weight Outlooks by Restriction of Diet and Sleep (WORDS) and Aerobic Treadmill Exercise and Metabolism (A-TEAM) studies, to examine the associations between sedentary time, PA of varying intensities, glycemic variability, and oxidative stress prior to either study's intervention. The WORDS study (ClinicalTrials.gov identifier: NCT02413866) was designed to examine the effects of chronic moderate sleep restriction on body composition and energy expenditure in individuals undergoing a hypocaloric dietary weight loss program, however, we will only be utilizing participants that completed baseline testing for the measurement of the outcomes pertinent to this dissertation, while the A-TEAM study (ClinicalTrials.gov identifier: NCT03162991) examined the effects of a moderate-intensity aerobic treadmill-based exercise intervention on glucose concentrations utilizing CGM technology in sedentary, overweight or obese adults. The third study utilized data collected from the A-TEAM study to examine the effect of a moderate-intensity aerobic treadmill-based exercise intervention on changes in sedentary time, PA, glycemic variability, and oxidative stress.

Study 1 Methodology- Critical Review on Current Literature Revolved around Sedentary Time and Physical Activity, Glycemic Variability, and Oxidative Stress in Adults

Purpose

This study addresses specific aim 1 and focuses on performing a critical review of literature on the topics of sedentary time and PA, glycemic variability, and oxidative stress. A critical review can be considered the summarization and evaluation of the ideas and information in an article. It predominantly expresses the writer's point of view in the light of what the writer has already obtained knowledge on the subject being discussed and what is acquired from related texts. Additionally, reviewing critically means thinking carefully and clearly and taking into consideration both the strengths and weaknesses in the material under review. This critical review focuses primarily on relevant research based on objectively measured sedentary time and PA, CGM assessed glycemic variability, and biomarkers of oxidative stress in adults.

Sub-Aim 1.1: To summarize and describe the known relationship between sedentary time and PA, glycemic variability, oxidative stress.

Sub-Aim 1.2: To summarize how acute exercise affects glycemic variability.

Sub-Aim 1.3: To summarize how repeated bouts of exercise and exercise training affects glycemic variability.

Study Design

This study is a critical review of the current literature surrounding sedentary time, PA, glycemic variability, and oxidative stress.

Research Design and Methods

Data Sources and Searches

To address sub-aim 1.1, which will be to establish what is known regarding the relationship between sedentary time and PA, glycemic variability, and oxidative stress, we plan to search the electronic databases PubMed, Google Scholar, Web of Science, BioMed Central, Cumulative Index of Nursing and Allied Health Literature (CINAHL), and SCOPUS databases up until December 2018. A variety of search terms were utilized for sedentary time, PA, continuous glucose monitoring, glycemic variability, and oxidative stress (outcome variables of interest). We plan to limit sedentary time and PA to objectively measured outcomes by validated accelerometry, but plan to include objectively assessed sedentary time and PA if yielded few search results. Glycemic variability will include only CGM assessed glycemic variability and oxidative stress will include any studies that examined the associations between biological markers of oxidative stress with glycemic variability. Studies will be included even if no relationship is found between sedentary time and PA and glycemic variability, or between glycemic variability and oxidative stress as research design and analysis of findings are of importance for this critical review. The search will be limited to adults; however, will include adults that are overweight or obese, have impaired glycemic states, diagnosed with type 1 or 2 diabetes, or cardiovascular disease risk factors in the presence or absence of medication which may affect outcomes of interest (population). These populations will be accounted for as age, body weight and health status, and medication usage may alter the outcome variables of interest, specifically glycemic variability and oxidative stress.

Similar to sub-aim 1.1, to address sub-aim 1.2, which will be to examine how the acute response to exercise affects the relationship on glycemic variability, we plan to search the electronic databases PubMed, Google Scholar, Web of Science, BioMed Central, CINAHL, and SCOPUS databases up until December 2018. Similar variables as of interest Sub Aim 1.1 will be utilized as search terms, including continuous glucose monitoring and glycemic variability; however, to examine the effect on the outcomes of interest, we will also perform a search to include acute aerobic exercise. Acute exercise will include monitored, clinically validated submaximal and maximal bout of exercise, including, but not limited to upper arm and lower leg ergometry, and treadmill based aerobic activity, and high-intensity interval training (HIIT) (treatments). We plan to limit glycemic variability to include only CGM assessed glycemic variability.

Lastly, to address sub-aim 1.3, which will be to examine how repeated bouts of exercise or exercise training affects glycemic variability, we will perform the same search as sub-aims 1.1 and 1.2, on the electronic databases PubMed, Google Scholar, Web of Science, BioMed Central, CINAHL, and SCOPUS databases up until December 2018. The same outcome variables of interest will be utilized as search terms, continuous glucose monitoring and glycemic variability as sub-aims 1.1 and 1.2; however, to expand upon findings from aim 1.2, we plan to examine the effect of repeated bouts of exercise and exercise training on glycemic variability, and a search including any repeated type of exercise, including short-term and chronic aerobic exercise interventions (treatment) on glycemic variability. This includes standardized or individualized and monitored exercise interventions, including HIIT training, performed in adults that do not have any limitations in their ability to perform aerobic exercise or have any contraindications to

perform aerobic exercise based on the criteria established by the American College of Sports Medicine, unless under clinical supervision during the exercise intervention (American College of Sports Medicine 2017).

For sub-aims 1.1, 1.2, and 1.3, we will limit the date range to December 2018 and will not place any language restrictions. We will include any duration of acute submaximal and maximal bouts of exercise and will define the acute response as immediately post-exercise up to 48 hours post-exercise, as long as the exercise bout is monitored and clinically validated. If studies included longer acute-phase durations past 48 hours post-exercise, they will be evaluated and included accordingly. Additionally, we will include repeated exercise and exercise interventions of all lengths in duration as long as they meet the minimum guidelines established for being considered physically active by the American College of Sports Medicine (American College of Sports Medicine 2017). Our secondary search strategy will include scanning bibliographies of the retrieved articles and searching for unpublished studies using key trial registries (clinicaltrials.gov and https://www.isrctn.com/).

Data Extraction and Study Quality

Initial selection will be based on title and abstract. The original article will be obtained and subsequently determined if the research study met the inclusion criteria established for the critical review. One reviewer will select the study independently and, if unsure about the reliability of the study in question, will seek a second reviewer to aid in the decision to include or exclude the article in the critical review. If there is disagreement between the primary and secondary reviewer, a third reviewer will be selected to provide a resolution to the inclusion or exclusion of the article in question for

the critical review. Reasons for exclusion will be documented and are available from the authors upon request.

Even though not a systematic review, data extraction and quality assessment will be based on the template from the Centre for Review and Dissemination (CRD) for systematic review guidelines (Tables 3.1. and 3.2.) (Centre for Reviews and Dissemination 2009). Extracted data will include details of the population, treatment, outcome variables of interest, and areas of potential bias. Biases evaluated in randomized controlled trials included adequate sequence generation, follow-up and exclusion biases, and intention to treat analysis. In non-randomized control trials, we plan to evaluate any baseline differences in participants, biases in allocation, follow-up and exclusion, and if analytical method was specified in the study protocol.

Table 3.1. Article Evaluation and Data Extraction Form (Sub-Aim Aim 1.1)

Article Details

- Study ID (First Author Name and Publication Date)

- Title of the Article

- Country

- Language

- Database

Study Details

- Aim of the Study

- Study Design

- Setting of the Study

- Duration of the Study

Methods

- Inclusion and/or Exclusion Criteria

- Outcome Measures

- Outcome Measure Method

- Outcome Evaluation Method

- Statistical Analysis

Participant Characteristics

- Number Enrolled and Number Analyzed

Enrolled: _____

Analyzed: _____

- Age

- Sex Composition (M/F), n (%)

- Race Composition

- Weight and BMI

Results

- Primary Findings

- Additional Findings

Conclusions

- Areas of Bias: _____
- Strengths: _____
- Weaknesses: _____

Additional Comments

- Reason for Exclusion: _____

Table 3.2. Article Evaluation and Data Extraction Form (Sub-Aims 1.2 and 1.3)

Article Details

- Study ID (First Author Name and Publication Date)

- Title of the Article

- Country

- Language

- Database

Study Details

- Aim of the Study

- Study Design

- Setting of the Study

- Duration of the Study

Methods

- Inclusion and/or Exclusion Criteria

- Randomization (If More than One Treatment)

- Allocation

- Blinding

- Outcome Measures

- Outcome Measure Method

- Outcome Evaluation Method

- Statistical Analysis

- Notes

Participant Characteristics

- Number Enrolled, Number Randomized, and Number Analyzed

Enrolled: _____

Randomized: _____

Analyzed: _____

- Age

- Sex Composition (M/F), n (%)

- Race Composition

- Weight and BMI

- Noted Differences between Randomization Groups

- Intervention Type and Intervention Descriptions

- Control Type and Control Description (If a Control Group is Included)

- Follow-Up Description

Results

- Primary Findings

- Additional Findings

Conclusions

- Areas of Bias: _____
- Strengths: _____
- Weaknesses: _____

Additional Comments

- Reason for Exclusion: _____

Strengths and Limitations of Study 1

Overall, there is a lack of well-documented studies that examine the relationship between sedentary time and PA with glycemic variability and oxidative stress, with even less information regarding the effect of acute and chronic aerobic exercise on glycemic variability. This study aims to be among the first to critically examine the current literature available regarding sedentary time and PA with glycemic variability and oxidative stress and compile a critical review to examine the current known information and delineate the gaps in the literature that need to be addressed. Specifically, this critical review aims to be the first to address this relationship in adults in a non-treatment and completing a treatment procedure (acute aerobic exercise and chronic aerobic exercise intervention). This critical review plans to include information from multiple sources, including PubMed, Google Scholar, Web of Science, BioMed Central, CINAHL, and SCOPUS databases up until December 2018. Additionally, a variety of search terms for each outcome variable of interest will be utilized to fully assess the relationship between sedentary time, PA, glycemic variability, and oxidative stress, as well as how glycemic variability may be altered due to acute and chronic aerobic exercise. There are several limitations to this study. The first will the primary use of objectively assessed sedentary time and PA; however, subjectively assessed sedentary time and PA will be incorporated as a secondary measure to fully elucidate this relationship, but have been shown to often lack validity, reliability, and reproducibility (Prince SA, Adamo KB, Hamel ME, Hardt J, Gorber SC, & Tremblay M, 2008). Additionally, only compiling articles utilizing CGM assessed glycemic variability has an inherent limitation as other clinical measures of glycemic variability exist. CGM assessed glycemic variability will be utilized as opposed

to other clinical measurements, such as fasting glucose concentrations and the glucose response during a glucose challenged state, as they may not be truly representative of glycemic variability that occurs over an extended period of time, or in a "free-living" environment, which will allow for greater insight into glycemic health. Furthermore, the accuracy of CGM measurements have been validated with clinical measures, such as venous blood sampling, in normo-glycemic individuals (Akintola AA, Noordam R, Jansen SW, de Craen AJ, Ballieux BE, Cobbaert CM, Mooijaart SP, Pijl H, Westendorp RG, & van Heemst D, 2015). We plan to include studies that examined the relationship between sedentary time and PA, continuous glucose monitor assessed glycemic, and biological markers of oxidative stress, as well as discuss oxidative stress markers that may have no existing relationship with sedentary time and PA or glycemic variability and mechanisms as to why when appropriate. Additionally, as the primary outcome variables of interest aim to address the overall research question regarding how sedentary time and PA interact with glycemic variability, we plan to incorporate how glycemic variability is altered following an acute aerobic exercise bout, and chronic aerobic exercise intervention. Lastly, the inclusion of a adults diagnosed with cardiometabolic disease, such as diabetes or cardiovascular disease risk factors, is a limitation, but will allow for a meaningful observation as to how sedentary time and PA relate to glycemic variability and oxidative stress in non-clinical and clinical populations, as well as provide greater insight into the acute and chronic response to aerobic exercise. As previously stated, even the presence of overweight and obesity immediately predisposes individuals to impaired glucose metabolism and cardiovascular disease risk factors, which will allow us to examine the relationship between sedentary time and PA with glycemic variability and

oxidative stress in an at-risk, non-disease state population. Overall, we plan to ensure included studies did not incorporate an adolescent or aging population to limit the effect of age on the outcome variables of interest, as well as guarantee that all populations in the articles included in this critical review are, other than potentially have impaired cardiometabolic status, otherwise healthy, presented with no complications or contraindications to exercise as described by the American College of Sports Medicine (American College of Sports Medicine 2017), or were appropriately monitored by trained medical staff. Moreover, as this study seeks to be the first to examine the relationship between objectively measured sedentary time and PA with measures of CGM assessed glycemic variability and oxidative stress, future studies will be able to utilize this critical review as a basis for the development of interventions aimed to decrease sedentary time, increase PA, and improve measures of glycemic variability and oxidative stress in adults at an increased risk for the development of, or in the presence of, metabolic and cardiovascular disease.

Study 2 Methodology- Sedentary Time and Physical Activity's Association with Glycemic Variability and Oxidative Stress

Purpose

This study addresses Specific Aim #2, which was to examine the cross-sectional associations between sedentary time and PA, glycemic variability, and biological markers of oxidative stress in overweight or obese adults.

Sub-Aim 2.1: To examine the associations between daily sedentary time, glycemic variability, and oxidative stress.

Sub-Aim 2.2: To examine the associations between daily LPA and MVPA, glycemic variability, and oxidative stress.

Sub-Aim 2.3: To examine the associations between daily total PA, glycemic variability, and oxidative stress.

Hypothesis

Sedentary time will be positively associated with glycemic variability and oxidative stress, while PA of varying intensities and total PA will be negatively associated with glycemic variability and oxidative stress.

Study Design

This study utilized a cross-sectional design.

Study Population and Enrollment Process

For both the WORDS and A-TEAM studies, 28 participants were recruited from the Columbia, South Carolina metropolitan area. The WORDS study openly recruited from January 2015 to October 2016, while the A-TEAM study openly recruited from October 2017 to December 2018. Participants were required to be currently healthy, sedentary, overweight or obese adults (males and females), age 35-55 years, have $25 \leq BMI \leq 40$ kg/m^2, be weight stable (±2%) during the previous 3 months, have <120 minutes of resistance or endurance exercise per week during the previous 3 months, and for females, be eumenorrheic, or post-menopausal for ≥1 year. Also, potential participants self-reporting medical conditions (e.g. diabetes), cardiovascular diseases, chronic or recurrent respiratory conditions (e.g. uncontrolled asthma or chronic obstructive pulmonary disease), active cancer, and eating, or neurological disorders, medications that affect metabolism (e.g. thyroid medications, statins), psychological issues, including but not limited to untreated depression and attention deficit disorder, excessive caffeine use (>500 mg/day), smoking during the past year, pregnant or lactating

females, unwillingness to provide informed consent were additional criterion for exclusion from the study. All participants in the studies met in the same laboratory setting, with the same research staff, which conducted all pertinent tests towards this dissertation in the same manner during the time of recruitment through study termination.

Sedentary Time and Physical Activity

Sedentary time and PA will be measured for all participants at 7 consecutive days and all participants were instructed to continue their normal daily routine during monitoring. Participants wore the Sensewear Mini Armband on the left arm at the mid-point between the olecranon and acromion processes. Participants recorded when they removed the monitor for any activities that required water-based activities, such as showering/bathing or swimming. Participants were instructed to record times they removed and replaced the monitor if removed over the 7-day wear time period. Data will be considered valid for analysis if participants wore the monitor for at least 5 days including a weekend day, with a minimum wear time of 20 hours each day.

Sedentary time and PA levels will be assessed using manufacture provided software (BodyMedia Sensewear Version 7.0). The Sensewear Mini Armband provides objectively measured PA. Sedentary time will be set as <1.5 metabolic equivalents (METs) (excluding sleep time), light-intensity PA as 1.5≤3.0 METs, moderate-intensity PA as 3.0≤6.0 METs, and vigorous-intensity PA as ≥6.0 METs, moderate-to-vigorous-intensity PA as ≥3.0 METs, and total PA intensity as ≥1.5 METs. In addition to time spent sedentary and performing PA of varying intensity, total daily energy expenditure (TDEE) and physical activity energy expenditure (PAEE) for total PA (≥1.5 METs) and moderate-to-vigorous-intensity PA (≥3.0 METs) will be analyzed. Time spent sedentary

and performing PA, TDEE, and PAEE will be calculated for each valid day of wear time and will be analyzed as an average of those days. Estimated PA time and energy expenditure during non-wear times will be excluded from analysis.

Glycemic Variability

Glycemic variability will be assessed by a CGM (Dexcom G4 Platinum Professional, San Diego, CA, USA). Participants had a sensor inserted under the skin on the preferred side of the abdomen, approximately 2 cm to the side of the umbilicus, and were required to carry a recording device for 7 consecutive days. The participants were trained to manually perform a capillary blood measurement (fingerstick) using a provided glucometer twice a day during wear time per manufacturer's instructions. The CGM device was blinded so that participants could not see the live readings to deter any alterations in diet, PA, or general lifestyle. Data was considered valid for analysis if participants wore the monitor for 5 days including a weekend day, with a minimum of 20 hours' data each day.

Software provided by the manufacturer (Dexcom Studio 12.0.4.6) was used to download and export the CGM data to Excel datafiles. The data were assessed in 5-minute intervals per 24-hour period. CGM data was transferred into and glycemic variability was analyzed per day using the EasyGV Version 9.0.R2, which is an Excel-enabled workbook that utilizes macros to calculate glycemic variability (University of Oxford, Oxford, England, UK). The MAGE and the CONGA-4 will be calculated and utilized as measurements of inter- and intra-day glycemic variability for each valid day of wear time and will be analyzed as an average of those days. Additionally, day-to-day

variability will be assessed as the standard deviation and coefficient of variation between the valid days analyzed.

Oxidative Stress

Oxidative stress will be assessed in serum (n=20; WORDS study) and plasma (n=8; A-TEAM study) from all participants who completed the studies and had fasting venous blood samples available at the end of the 7-day monitoring period. Prior to analysis, as samples are collected, they will be centrifuged at 3,000 rpm at 4 degrees Celsius for 20 minutes and stored at -80 degrees Celsius until all samples are collected. Once all sample were collected and ready for analysis, samples will be thawed and re-centrifuged to separate any particulate. Two biological biomarkers or oxidative stress, nitric oxide and myeloperoxidase, will be measured using two separate enzyme-linked immunoabsorbant assays (ELISA). The nitric oxide ELISA kit (ThermoFicher Scientific, Waltham, MA) will be quantitatively determined by the concentrations of nitrate and nitrite in serum and plasma samples. This ELISA utilized the enzyme nitrate reductase to convert nitrate to nitrite, which was then detected as a colored azo dye product of the Griess reaction which absorbs light at 540 nm. The interaction of nitrate and nitrite concentrations measured will determine the concentration of nitric oxide in both serum and plasma (Fareed D, Tqbal O, Tobu M, Hoppensteadt DA, & Fareed J, 2004). Proper sample dilution and preparation will allow for a low percent coefficient of variation, and both human serum and plasma samples have ≥90% sample recovery for both nitrite and nitrate concentrations which will be utilized to calculate nitric oxide, alleviating potential error due to intra-assay and inter-assay variability per manufacturer's product information sheet. The myeloperoxidase serum/plasma ELISA kit (Eagle Biosciences, Inc., Nashua,

NH) will be utilized to quantify the determination of myeloperoxidase utilizing a two-site "sandwich" technique that binds to different epitopes of myeloperoxidase. Antibodies bind to myeloperoxidase and, after several incubation periods and plate washes, will be ready to analyze by detecting the immunocomplex and the absorbency of the sample. Intra-assay and inter-assay variability for concentrations of myeloperoxidase were comparable in serum and plasma utilized per manufacturer's product information sheet in which they measured two sample extracts in a single assay with twelve replicate determinations for intra-assay variability, and by measuring two controls in duplicate in six individual assays.

Fasting Glucose Concentration and Oral Glucose Tolerance Test

For the WORDS study, on the final day of 7-day CGM monitoring period each participant completed an OGTT at baseline and post-intervention. Following an overnight fast (~12 hours other than water), participants reported to an approved clinical site and performed a venous blood sample collection in which a catheter was inserted into the antecubital space of the arm. Time was recorded when am initial venous blood sample was collected to establish fasting blood glucose time point. Following the venous blood collection, participants were then instructed to consume a standard 10-ounce, 75-gram glucose infused drink (Azer Scienmtific, Morgantown, PA) within 5 minutes of the venous blood collection and time consumption completed was recorded and utilized as time point zero (0-). Every 30-minute interval was then recorded until 2 hours was complete (30-,60-,90-,120-minutes post-consumption), which is the standard procedure for a 75-gram OGTT (Institute for Quality and Efficiency in Healthcare 2011). During the 2-hour OGTT, participants were instructed to limit their movement, unless necessary,

to limit the impact of muscle contraction on glucose concentrations. All venous blood samples were collected into 1.5 mL centrifuge tubes and placed on ice until completion of the 2-hour OGTT and subsequently stored at -80 degrees Celsius until all samples are collected. Fasting and 30-,60-,90-, and 120-minute post-consumption concentrations of blood glucose were analyzed utilizing a YSI 2300 STAT Plus (YSI Life Sciences, Yellow Spring, OH), which was calibrated according to manufacturer instruction prior to analysis.

For the A-TEAM study, on the final day of 7-day CGM monitoring period, while participants were still wearing the CGM monitor, each participant completed an OGTT at baseline and post-intervention. Following an overnight fast (~12 hours other than water), participants reported to an approved clinical site and performed a venous blood sample collection. Time was recorded and matched with the CGM to the venous blood collection to establish fasting blood glucose time point. Following the venous blood collection, participants were then instructed to consume a standard 10-ounce, 75-gram glucose infused drink (Azer Scienmtific, Morgantown, PA) within 5 minutes of the venous blood collection and time consumption completed was recorded and utilized as time point zero (0-). Every 30-minute interval was then recorded until 2 hours was complete (30-,60-,90-,120-minutes post-consumption), which is the standard procedure for a 75-gram OGTT (Institute for Quality and Efficiency in Healthcare 2011). During the 2-hour OGTT, participants were instructed to limit their movement, unless necessary, to limit the impact of muscle contraction on glucose concentrations. As CGM assessed glucose concentrations have been validated with venous blood glucose concentrations (Kovatchev

B, Anderson S, Heinemann L, & Clarke W, 2008), no subsequent blood draws were performed at each time point after fasting.

Software provided by the manufacturer (Dexcom Studio 12.0.4.6) was used to download and export the CGM data to Excel datafiles. Time points recorded during the OGTT were identified and matched with exported CGM data for time point 0 and subsequent 30-,60-,90-,120-minute post-consumption time points. If any of the time points fell between CGM concentration readings, as the CGM assesses every 5 minutes, the average between the previous and following CGM concentration readings were calculated. Additionally, OGTT area under the curve (AUC) was calculated utilizing the equation Glucose AUC=$1/2\times30\times(y_{0\text{-}}+2y_{30\text{-}}+2y_{60\text{-}}+2y_{90\text{-}}+y_{120\text{-}})$, where y represents glucose concentration at the different time points (Tai MM 1994).

Statistical Analysis

Descriptive statistics were calculated and reported as means and standard deviations. To address all sub-aims, the average of valid daily sedentary time, and LPA, MPA, VPA, and MVPA, total PA time, TDEE, and PAEE (≥1.5 and ≥3.0 METs) will be calculated. Furthermore, the average of valid daily glycemic variability assessed via MAGE, CONGA-4, and MODD will be calculated, and fasting concentrations of nitric oxide and myeloperoxidase will be measured. To address sub-aim 2.1, Pearson product correlations between sedentary time and MAGE, CONGA-4, and MODD, and nitric oxide and myeloperoxidase will be performed. To address sub-aim 2.2, Pearson product correlations will be performed between LPA and MVPA time, TDEE, and PAEE (≥3.0 METs) between both measures MAGE and CONGA-4 and nitric oxide and myeloperoxidase. To address sub-aim 2.3, Pearson product correlations will be

performed between total PA time and PAEE (≥1.5 METs) and MAGE and CONGA-4, and nitric oxide and myeloperoxidase. Furthermore, to address the relationship between daily glycemic variability and oxidative stress, Pearson product correlations will be performed between both measures of glycemic variability and oxidative stress (i.e. MAGE and nitric oxide, MAGE and myeloperoxidase, CONGA-4 and nitric oxide, and CONGA-4 and myeloperoxidase). Additionally, for sub-aims 2.1 and 2.2, and 2.3, age, sex, race, and/or BMI will be adjusted for to examine the role of participant characteristics on the associations between measures of glycemic variability and oxidative stress. Lastly, as we are including participants from two different studies, with slightly different methodology, we plan to adjust for study involvement (WORDS and A-TEAM) for all three sub-aims to assess whether sample type may affect the associations of interest. Lastly, to assess potential day-to-day variability of sedentary and PA measures and variation in glycemic variability on their relationship with oxidative stress, Pearson product correlations will be performed between the standard deviation and coefficient of variation for sedentary and PA measures and measures of glycemic variability with fasting oxidative stress concentrations.

Statistical Power

There is currently no info in the literature examining the relationship between sedentary time, PA of varying intensities, and measures of glycemic variability and oxidative stress. Therefore, power analysis calculations were performed using G*Power 3.0.10 and found that when testing for the sensitivity of a required significant correlation, 28 participants would allow for 80% power with a medium- to large-correlation (-1.0--0.3; 0.3-1.0) with alpha set to 0.05 when testing for the correlation between the outcome

variables of interest, sedentary time and PA of varying intensities with glycemic variability and oxidative stress. As this is the first study to examine the relationship between daily sedentary time, PA of varying intensities, measures of glycemic variability, and fasting concentrations of biomarkers associated with oxidative stress, results will be utilized to provide evidence for power calculations for future studies.

Strengths and Limitations of Study 2

This study is among the first to examine the relationship between sedentary time, PA of varying intensities, glycemic variability, and oxidative stress. The use of CGM technology to assess glycemic variability is another strength, which allows for the observation of a "free-living" condition as opposed to standard clinical measures. The strengths of this study also included the use of trained staff for the placement and implementation of not only the Sensewear Mini Armband, but also the Dexcom CGM device, trained phlebotomists completed the blood collection and processing prior to analysis of oxidative stress biomarkers, nitric oxide and myeloperoxidase. There are limitations for this proposed study, including the demographics of the population, which is considered a convenience sample, and only makes the results generalizable to sedentary, overweight or obese individuals that are between the ages of 35 and 55 years. Additionally, undiagnosed diabetics were not excluded from the study, which may influence the results. In order to limit this, no participants were currently taking diabetic medications during measurement time period. Following completion of the 7-day monitoring period, each participant's CGM glucose levels were analyzed and none of the completers fell into the prescribed diabetic range (fasting blood glucose ≥126 mg/dL). Furthermore, diet was not fully taken into account, as only self-report dietary intake was

provided, which has been known to be unreliable. In order to limit this, we recommended that each participant attempt to maintain their daily dietary routines during the 7-day monitoring process. However, not controlling for diet may also be considered a strength as their "normal" dietary patterns may provide an insightful examination into the "free-living" glycemic variability observed. For females, current cycle of menstruation was not taken into account, as current cycle in the menstrual period may influence hormones associated with glucose metabolism and cardiovascular outcomes. Past research has found that both glucose metabolism and oxidative stress may be altered during various phases of the menstrual cycle (Widom B, Diamond MP, Simonson DC, & 1992; Browne RW, Bloom MS, Schisterman EF, Hovey K, Trevisan M, Wu C, Liu A, & Wactawski-Wende J, 2008). As we have no way of retroactively examining menstrual cycle in female participants, this limitation will persist, but provide future directions for the consideration of menstrual cycle phase in subsequent research studies.

STUDY 3 METHODOLOGY-THE IMPACT OF AEROBIC EXERCISE TRAINING ON PA, GLYCEMIC VARIABILITY, AND OXIDATIVE STRESS

Purpose

This study addressed Specific Aim #3, which was to examine the effect of a 12-week aerobic exercise intervention on glycemic variability and oxidative stress.

Sub-Aim 3.1: To examine the effect of a 12-week aerobic exercise intervention on glycemic variability.

Sub-Aim 3.2*:* To examine the effect of a 12-week aerobic exercise intervention on oxidative stress.

Hypothesis

An aerobic treadmill-based intervention aimed at increasing structured PA through aerobic-exercise training will positively impact and improve measures of glycemic variability through reduction of glycemic variability assessed as MAGE and CONGA-4, and measured biomarkers of oxidative stress by increasing concentrations of circulating nitric oxide and decreasing circulating concentrations of myeloperoxidase.

Study Design

This study utilized an interventional design.

Study Population and Enrollment Process

For the A-TEAM study, eight participants were recruited from the Columbia, South Carolina metropolitan area between October 2017 and December 2018. Potential participants were required to be currently healthy, sedentary, overweight or obese adults (males and females), age 35-55 years, have $25 \leq BMI \leq 40$ kg/m^2, be weight stable (±2%) during the previous 3 months, have <120 minutes of reported resistance or endurance exercise per week during the previous 3 months, and for females, be eumenorrheic, or post-menopausal for ≥1 year. Also, potential participants self-reporting medical conditions (e.g. diabetes), cardiovascular diseases, chronic or recurrent respiratory conditions (e.g. uncontrolled asthma or chronic obstructive pulmonary disease), active cancer, and eating, or neurological disorders, medications that affect metabolism (e.g. thyroid medications, statins), psychological issues, including but not limited to untreated depression and attention deficit disorder, excessive caffeine use (>500 mg/day), smoking during the past year, pregnant or lactating females, and unwillingness to provide informed consent were additional criterion for exclusion from the study.

All participants answered a medical history questionnaire and underwent a series of medical tests to identify and medical conditions that could potentially interfere with participation in the exercise intervention, which included resting blood pressure, resting electrocardiogram (ECG), graded exercise test, and CGM assessed fasting glucose concentrations. If potential participants are found to fall in the range of diabetic without prior diagnosis following the week wearing the CGM, they were provided the daily glucose concentrations obtained from the CGM and instructed to see a physician.

Study Intervention

Participants completed a 12-week moderate-intensity aerobic treadmill-based exercise intervention. Participants walked 3 times a week on treadmills in a controlled research facility under trained supervision. The exercise volume was achieved by varying the duration, speed, and grade to reach each participant's energy expenditure goal according to body weight (10-12 kcal per kg of body weight each week). Weekly energy expenditure was determined by multiplying each participant's body weight by the energy expenditure goal (10-12 kcal per kg of body weight each week) and was closely monitored throughout the 12-week intervention. Adherence to the exercise prescription (weekly energy expenditure of 10-12 kcal per kg of body weight each week) was monitored by calculating the amount of energy expenditure each exercise session using the standard American College of Sports Medicine formula: {0.1×(speed[miles per hour] ×26.8)+1.8×(speed[miles per hour] ×26.8)×grade(%)+3.5}×body weight(kg)÷5(L per minute)×time(minutes) (American College of Sports Medicine 2017). 12 weeks of aerobic exercise training has been extensively studied and is considered an appropriate duration for physiological adaptations to aerobic exercise to occur (Ho SS, Dhaliwal SS,

Hills AP, & Pal S, 2012). The prescribed training volume, 10-12 kcal per kg of body weight each week, is considered a higher volume of aerobic exercise energy expenditure compared to past guidelines, which suggest 8 kcal per kg of body weight each week should elicit physiological alterations; however, as that study design also noted physiological changes at 12 kcal per kg of body weight each week, and utilized a 6 month intervention, 10-12 kcal per kg of body weight each week for 12 weeks of moderate-intensity aerobic exercise was assigned (Morss GM, Jordan AN, Skinner JS, Dunn AL, Church TS, Earnest CP, Kampert JB, Jurca R, & Blair SN, 2004; Sisson SB, Katzmarzyk, Earnest CP, Bouchard C, Blair SN, & Church TS, 2009; Rosenkilde M, Reichkendler MH, Auerbach P, Bonne TC, Sjödin A, Ploug T, & Stallknecht BM, 2015).

Due to the physically inactive state of the participants, the exercise intensity and weekly energy expenditure was gradually increased to reduce risk of injury. Training intensity increased during the first 4 weeks of aerobic treadmill-based exercise intervention until the target level of 50-55% of participant's heart rate reserve (HRR) was met, which was determined during the baseline graded exercise test. Participants began at a weekly energy expenditure of 6-8 kcal/kg of body weight during the first week of the intervention and then progressed until their weekly energy expenditure (10-12 kcal/kg of body weight) was attained by week 4. Each exercise session began and ended with a 3-minute warm-up and cool-down. Heart rate (HR) monitors (FT1; Polar, Lake Success, NY, USA) were worn to monitor exercise intensity continuously throughout each exercise session and HR was recorded every five minutes. If HR monitors were unable to detect HR, manual palpation at the radial artery was measured for 30-60 seconds. Blood

pressure was measured before, during warm-up, at the mid-point of the exercise session, during cool-down, and following each exercise session.

Compliance to the weekly weigh-ins and prescribed exercise intervention (frequency, intensity, and duration) for each participant was reviewed weekly and any participant missing an exercise session without notifying study personnel was contacted via phone or e-mail to reschedule and encourage further attendance.

Glycemic Variability

Glycemic variability will be assessed by a CGM (Dexcom G4 Platinum Professional, San Diego, CA, USA). Participants had a sensor inserted under the skin on the preferred side of the abdomen, approximately 2 cm to the side of the umbilicus, and were required to carry a recording device for 7 consecutive days at baseline and end-intervention. The participants were trained to manually perform a capillary blood measurement (fingerstick) using a provided glucometer twice a day during wear time per manufacturer's instructions. The CGM device was blinded so that participants could not see the live readings to deter any alterations in diet, PA, or general lifestyle. Data was considered valid for analysis if participants wore the monitor for 5 days including a weekend day, with a minimum of 20 hours' data each day.

Software provided by the manufacturer (Dexcom Studio 12.0.4.6) was used to download and export the CGM data to Excel datafiles. The data were assessed in 5-minute intervals per 24-hour period. CGM data was transferred into and glycemic variability was analyzed per day using the EasyGV Version 9.0.R2, which is an Excel-enabled workbook that utilizes macros to calculate glycemic variability (University of Oxford, Oxford, England, UK). The MAGE and the CONGA-4 will be calculated and

utilized as measurements of intra-day glycemic variability for each valid day of wear time and will be analyzed as an average of those days. Additionally, day-to-day variability will be assessed as the standard deviation and coefficient of variation between the valid days analyzed.

Oxidative Stress

Oxidative stress will be assessed in plasma (n=8) from all participants who completed the studies and had fasting venous blood samples available at the end of the 7-day monitoring period at baseline and end-intervention. Prior to analysis, as samples are collected, they will be centrifuged at 3,000 rpm at 4 degrees Celsius for 20 minutes and stored at -80 degrees Celsius until all samples are collected. Once all sample were collected and ready for analysis, samples will be thawed and re-centrifuged to separate any particulate. Two biological biomarkers or oxidative stress, nitric oxide and myeloperoxidase, will be measured using two separate enzyme-linked immunoabsorbant assays (ELISA). The nitric oxide ELISA kit (ThermoFicher Scientific, Waltham, MA) will be quantitatively determined by the concentrations of nitrate and nitrite in serum and plasma samples. This ELISA utilized the enzyme nitrate reductase to convert nitrate to nitrite, which was then detected as a colored azo dye product of the Griess reaction which absorbs light at 540 nm. The interaction of nitrate and nitrite concentrations measured will determine the concentration of nitric oxide in both serum and plasma (Fareed D, Tqbal O, Tobu M, Hoppensteadt DA, & Fareed J, 2004). Proper sample dilution and preparation will allow for a low percent coefficient of variation, and both human serum and plasma samples have ≥90% sample recovery for both nitrite and nitrate concentrations which will be utilized to calculate nitric oxide, alleviating potential error

due to intra-assay and inter-assay variability per manufacturer's product information sheet. The myeloperoxidase serum/plasma ELISA kit (Eagle Biosciences, Inc., Nashua, NH) will be utilized to quantify the determination of myeloperoxidase utilizing a two-site "sandwich" technique that binds to different epitopes of myeloperoxidase. Antibodies bind to myeloperoxidase, after several incubation periods and plate washes, will be ready to analyze by detecting the immunocomplex and the absorbency of the sample. Intra-assay and inter-assay variability for concentrations of myeloperoxidase were comparable in serum and plasma utilized per manufacturer's product information sheet in which they measured two sample extracts in a single assay with twelve replicate determinations for intra-assay variability, and by measuring two controls in duplicate in six individual assays for inter-assay variability. Additionally, all samples were collected within 10 to 14 days following the final bout of exercise to limit the effect of acute exercise on markers of oxidative stress, as well as within a time frame that does not allow for the effect of detraining on the markers of oxidative stress utilized in this study (Maeda S, Miyauchi T, Kakiyama T, Sugawara J, Iemitsu M, Irukayama-Tombe Y, Murakami H, Kumagi Y, Kuno S, Matsuda M, 2001).

Cardiorespiratory Fitness (CRF)

CRF for all participants was measured at baseline and end-intervention using a maximal graded exercise test. A ramped medium protocol was determined to be ideal for these participants as it is an incremental protocol where speed and grade increase every 30-60 seconds until each participant reached volitional fatigue. Volume of oxygen consumed ($\dot{V}O_2$) via a metabolic cart (True Max 2400; ParvoMedics, Sandy, UT, USA) and heart rate via standard 12-lead electrocardiogram (Q-Stress®; Cardiac Science,

Bothell, WA, USA) were monitored continuously during the progression of the test. Blood pressure was measured, and rating of perceived exertion was obtained every two-minutes during the test. Two of four generally recognizable criteria for the test to be considered satisfactory needed to be achieved: a respiratory exchange ratio greater than or equal to 1.10; a rating of perceived exertion greater than or equal to 17 on the Borg scale ranging from 6-20; achieving a maximum heart rate greater than 90% age-predicted maximum heart rate (220-age); and/or a plateau in V̇o2. Peak oxygen consumption ($\dot{V}O_{2peak}$) was determined by the highest 30-second average $\dot{V}O_2$ value measured during the test.

Sedentary Time and Physical Activity

Sedentary time and PA will be measured for all participants for 7 consecutive days at baseline and post-intervention and all participants were instructed to continue their normal daily routine during monitoring. Participants wore the Sensewear Mini Armband on the left arm at the mid-point between the olecranon and acromion processes. Participants recorded when they removed the monitor for any activities that required water-based activities, such as showering/bathing or swimming. Participants were instructed to record times they removed and replaced the monitor if removed over the 7-day wear time period. Data will be considered valid for analysis if participants wore the monitor for at least 5 days including a weekend day, with a minimum wear time of 20 hours each day.

Sedentary time and PA levels will be assessed using manufacture provided software (BodyMedia Sensewear Version 7.0). The Sensewear Mini Armband provides objectively measured PA. Sedentary time will be set as <1.5 metabolic equivalents

(METs) (excluding sleep time), light-intensity PA as 1.5≤3.0 METs, moderate-intensity PA as 3.0≤6.0 METs, and vigorous-intensity PA as ≥6.0 METs, moderate-to-vigorous-intensity PA as ≥3.0 METs, and total PA intensity as ≥1.5 METs. In addition to time spent sedentary and performing PA of varying intensity, total daily energy expenditure (TDEE) and physical activity energy expenditure (PAEE) for total PA (≥1.5 METs) and moderate-to-vigorous-intensity PA (≥3.0 METs) will be analyzed. Time spent sedentary and performing PA, TDEE, and PAEE will be calculated for each valid day of wear time and will be analyzed as an average of those days. Estimated PA time and energy expenditure during non-wear times will be excluded from analysis.

Fasting Glucose Concentration and Oral Glucose Tolerance Test

On the final day of 7-day CGM monitoring period, while participants were still wearing the CGM monitor, each participant completed an OGTT at baseline and post-intervention. Following an overnight fast (~12 hours other than water), participants reported to an approved clinical site and performed a venous blood sample collection. Time was recorded and matched with the CGM to the venous blood collection to establish fasting blood glucose time point. Following the venous blood collection, participants were then instructed to consume a standard 10-ounce, 75-gram glucose infused drink (Azer Scienmtific, Morgantown, PA) within 5 minutes of the venous blood collection and time consumption completed was recorded and utilized as time point zero (0-). Every 30-minutes interval was then recorded until 2 hours was complete (30-,60-,90-,120-minutes post-consumption), which is the standard procedure for a 75-gram OGTT (Institute for Quality and Efficiency in Healthcare 2011). During the 2-hour OGTT, participants were instructed to limit their movement, unless necessary, to limit the

impact of muscle contraction on glucose concentrations. As CGM assessed glucose concentrations have been validated with venous blood glucose concentrations (Kovatchev B, Anderson S, Heinemann L, & Clarke W, 2008), no subsequent blood draws were performed at each time point after fasting.

Software provided by the manufacturer (Dexcom Studio 12.0.4.6) was used to download and export the CGM data to Excel datafiles. Time points recorded during the OGTT were identified and matched with exported CGM data for time point 0 and subsequent 30-,60-,90-,120-minute post-consumption time points. If any of the time points fell between CGM concentration readings, as the CGM assesses every 5 minutes, the average between the previous and following CGM concentration readings were calculated. Additionally, OGTT area under the curve (AUC) was calculated utilizing the equation Glucose AUC=$1/2\times30\times(y_{0\text{-}}+2y_{30\text{-}}+2y_{60\text{-}}+2y_{90\text{-}}+y_{120\text{-}})$, where y represents glucose concentration at the different time points (Tai MM 1994).

Caloric Intake Recording

Caloric intake will be recorded for all participants for 7 consecutive days at baseline and post-intervention and all participants were instructed to continue their normal daily dietary routine during this time. All participants self-reported caloric consumption, including any calorie containing beverages or snacks, for one week during baseline and post-intervention testing. They were instructed on using the MyFitnessPal application (MyFitnessPal, Inc.) that is available on smartphone devices or computer based. If a smartphone or computer was unavailable, participants were provided a self-report paper form to record any calorie containing food or drink consumed, including portion size, calories, and macronutrient breakdown (carbohydrate, protein, and fat)

utilizing The Calorie King® Calorie Counter (Borushek A 2015). Caloric intake and macronutrient breakdown will be calculated for each valid recording day and will be analyzed as an average of those valid days.

Statistical Analysis

Baseline descriptive statistics will be calculated and reported as means and standard deviations. Change values will be calculated by subtracting baseline values from post-intervention values. Paired sample t-tests will be utilized to determine how participant characteristics, clinical measurements of glycemic health, fitness, and lifestyle factors changed from baseline to post-intervention (body weight, CRF, sedentary and PA measures, fasting glucose concentration, 120-minute OGTT concentration, glucose AUC, and caloric intake). To address sub-aim 3.1, a repeated measure analysis of variance (ANOVA) will be performed from baseline to end-intervention for the MAGE and CONGA-4 to establish changes in glycemic variability. To address sub-aim 3.2, similar to sub-aim 3.1, a repeated measure ANOVA will also be performed to analyze changes in nitric oxide and myeloperoxidase. For each sub-aim, separate models will be performed for both measures of glycemic variability and oxidative stress. Additionally, for sub-aim 3.1 and 3.2, change in body weight, sedentary and PA measures, CRF, fasting glucose concentration, 120-minute OGTT concentration, glucose AUC, and/or caloric intake will be adjusted for to examine the role of participant characteristics, as well as clinical measurements of glycemic health, fitness, and lifestyle factors as covariates on the changes in measures of glycemic variability and oxidative stress. Lastly, to explore a potential relationship between glycemic variability and oxidative stress following aerobic exercise training, Pearson product correlation will be performed between change in

measurements of glycemic variability and change in measurements of oxidative stress, which will also be adjusted for using the previously mentioned variables as covariates.

Statistical Power

There is currently no literature examining the effect of a chronic aerobic treadmill-based exercise intervention on changes in glycemic variability and oxidative stress, or the associations that exist between these two variables. To date, one study has examined the influence of aerobic exercise, 7 days of structured aerobic exercise, on glycemic variability and control assessed by CGM technology in adults with type 2 diabetes mellitus not using exogenous insulin (Mikus CR, Oberlin DJ, Libla J, Boyle LJ, & Thyfault JP, 2012). Therefore, this study was utilized for sample size estimations. Sedentary, overweight or obese adults diagnosed with type 2 debates mellitus (n=13; age=53.0±2.0 years; BMI=34.1±1.3 kg/m^2) underwent glycemic variability and control assessment utilizing CGM technology during 3 days of habitual activity and during the final 3 days of a 7 day aerobic exercise training program, which consisted of 60 minutes of supervised exercise for 7 consecutive days at 60-75% HRR, and found that "free-living" glycemic excursions were lower for the 3 days while completing the aerobic exercise program compared to the 3 days of habitual activity (maximum blood glucose: 13.6±1.2 mmol/l to 10.9±0.8 mmol/l, $p<0.01$; Δmin–max blood glucose:10.0±1.1 mmol/l to 6.9±0.7 mmol/l, $p<0.01$; number of glucose excursion per day: 0.8±0.2 to 0.2±0.1, $p=0.02$). Additionally, another study that examined basal nitric oxide production in hypercholesterolemic patients (n=9; age=44.0±3.0; BMI=27.0±1.0 kg/m^2) undergoing 4 weeks of home-based cycle training 3 times per week at 65% $\dot{V}O_{2max}$ for 30 minutes each session found that basal nitric oxide production increased following training (ΔNOx

indicated net extraction=6.8±4.0 nmol/100mL/min) (Lewis TV, Dart AM, Chin-Dusting JPF, & Kingwell BA, 1999). Therefore, further power analysis calculations were performed using G*Power 3.0.10 and found that when testing for the sensitivity of required effect size, 8 participants would allow for 20% power with a small- to medium-effect size (0.1-0.3) with alpha set to 0.05 when testing for the difference between two dependent means, baseline compared to end-intervention for measures of glycemic variability and oxidative stress. Thus, this study will also serve to provide additional resources for future effect size examining alterations in measures of glycemic variability and oxidative stress following an aerobic treadmill-based exercise intervention in sedentary, overweight or obese adults.

Strengths and Limitations of Study 3

This study is among the first to examine the effects of exercise on glycemic variability and how glycemic variability relates to oxidative stress both before and after becoming physically active. The strengths of this study included the use of center-based, individually designed aerobic treadmill-based intervention in overweight or obese individuals that was intended to not only meet a desired intensity, but volume of exercise quantified by estimated caloric expenditure each week. In addition, each exercise session was performed under supervision in a laboratory setting which allowed for adherence to exercise protocol to be monitored. The use of CGM technology to assess glycemic variability is another strength. There are limitations for this proposed study, including the demographics of the population, which only makes the results generalizable to individuals that are between the ages of 35 and 55 years. Additionally, undiagnosed diabetics were not excluded from the study, which may influence the results. In order to

limit this, each participant's CGM glucose levels were analyzed prior to the intervention, and none of the completers fell into the prescribed diabetic range. Furthermore, diet was not fully taken into account, as only self-report dietary intake was provided at baseline and end-intervention, which has been known to be unreliable. In order to limit this, we recommended that each participant attempt to maintain their daily dietary routines throughout the intervention and not participate in any other interventions that may have affected their weight. For females, current cycle of menstruation was not taken into account, as current cycle in the menstrual period may influence hormones associated with glucose metabolism and cardiovascular outcomes. Past research has found that both glucose metabolism and oxidative stress may be altered during various phases of the menstrual cycle (Widom B, Diamond MP, Simonson DC, 1992; Browne RW, Bloom MS, Schisterman EF, Hovey K, Trevisan M, Wu C, Liu A, Wactawski-Wende J, 2008). As we have no way of retroactively examining menstrual cycle in female participants, this limitation will persist, but provide future directions for the consideration of menstrual cycle phase in subsequent research studies. Lastly, the small sample size (n=8) may limit the statistical power needed to detect a true effect size of the outcomes of interest. However, this study was designed to add to the mostly non-existent literature and aid in the prediction of future effect sizes for studies wishing to examine the effect of exercise on glycemic variability and oxidative stress.

CHAPTER 4

MANUSCRIPT 1-GLYCEMIC VARIABILITY: IMPORTANCE, RELATIONSHIP WITH PHYSICAL ACTIVITY, AND THE INFLUENCE OF EXERCISE-A CRITICAL REVIEW OF THE LITERATURE[1]

[1]Sparks, JR, Kishman, EE, Sarzynski, MA, Davis, JM, Grandjean, PW, & Wang, X. To be submitted to *Scientific Journal of Research and Reviews*

ABSTRACT

Glycemic variability has recently been thought of as a potentially more sensitive assessment of glycemic health as opposed to traditional clinical measures of glucose metabolism and glucose tolerance. The ability for glycemic variability to consider all glucose concentrations in a given distribution and account for the oscillations that occur in these distributions provide clinicians with more in-depth insight into how individuals regulate and/or maintain their glycemic health. With the advancement of continuous glucose monitoring (CGM) technology to allow examination of real-time, free-living glucose concentrations over a single or multiple days, more reliable assessment and treatment of dysregulated glycemic health, specifically in impaired glucose tolerant and type 1 and type 2 diabetic adults, is possible. CGM assessment along with lifestyle management techniques implemented to influence glucose concentrations, such as sedentary behavior and physical activity, including structured physical activity, known as exercise, allow for a greater perspective to be established linking lifestyle factors with glycemic health. Therefore, the aim of this review is to critically evaluate and provide evidence regarding the importance of glycemic variability, how glycemic variability is measured, the relationship glycemic variability possesses with sedentary time and physical activity, the influence of a single bout or repeated bouts of exercise, as well as exercise training on glycemic variability in non-diabetic adults, and adults diagnosed with type 1 or type 2 diabetes. Additionally, this review plans to provide strengths and limitations of the studies discussed, which will allow insight into future design of research studies aimed to examine glycemic variability as a primary outcome of interest.

INTRODUCTION

Glycemic variability has been considered a novel and sensitive measure of glycemic health in addition to clinical assessment of glucose metabolism and tolerance (Monnier L, Colette C, & Owens DR, 2008). Glycemic variability accounts for glucose fluctuations throughout the day or during specified durations (Hirsch IB & Brownlee M, 2005). Glycemic variability has been observed to be greater in overweight to obese adults, as well as in adults diagnosed with type 1 and type 2 diabetes, compared to healthy, normal weight adults (Buscemi S, Cosentino L, Rosafio G, Morgana M, Mattina A, Sprini D, Verga S, & Rini GB, 2013). As glycemic variability has been related to risk of the development of cardiovascular disease risk factors, specifically increased endothelium-derived oxidative stress (Saisho Y 2014), the clinical implications for the assessment of glycemic variability have become of great importance.

The use of continuous glucose monitoring (CGM) allows for the inclusion and evaluation of all glucose concentration oscillations over an extended monitoring period (Standl E, Schnell O, & Ceriello A, 2011). CGM assesses real-time glucose concentrations and instantaneously allows for users and practitioners to make more informed decisions throughout the day on how to balance self-management techniques to maintain glycemic control (Hirsch IB, Armstrong D, Bergenstal RM, Buckinghom B, Childs BP, Clarke WL, & Wolpert H, 2008). In addition, CGM measurements can take place every few minutes, over multiple hours throughout the day, for several days to aid in assessment of habitual glycemic health (Vincze G, Barner JC, & Lopez D, 2004). CGM works through a sensor inserted in subcutaneous tissue and measures interstitial fluid concentration of glucose, which circulates in the fluid between cells (Nielsen JK,

Djurhuus CB, Gravholt CH, Carus AC, Granild-Jensen J, Orskov H, & Christiansen JS, 2005). CGM-measured glucose concentrations have been validated against venous blood glucose concentrations (Bailey TS 2017). Even though a delay may occur between measurements of glucose concentrations, with the delay occurring in interstitial fluid assessment compared to venous blood assessment, this delay has been shown to be minimal and often varies marginally by individual (Garg SK, Voelmle M, & Gottlieb PA, 2010). Thus, when utilized in research, CGM measured glucose concentrations can aid in establishment of time spent at hypo-, hyper-, and euglycemia, and allows for the assessment of glycemic variability (Klonoff DC 2005).

Decreased sedentary time and increased physical activity of any intensity have been shown to be beneficial for overall health, specifically glycemic health (Høstmark AT, Ekeland GS, Beckstrøm AC, & Meen HD, 2006; Carnethon MR, Evans NS, Church TS, Lewis CE, Schreiner PJ Jacobs Jr DR, Sternfel B, & Sidney S, 2010; Nygaard H, Grindaker E, Rønnestad BR, Holmboe-Ottesen G, & Høstmark AT, 2017). Structured physical activity, commonly known as exercise, has been shown to have additional glycemic health benefits beyond non-exercise related physical activity (Boulé NG, Haddad E, & Kenny GP, 2001). Recently, it has been suggested that even a single bout of exercise or repeated bouts of exercise improve glycemic variability in healthy and metabolically compromised adults, with potential improvements following exercise training noted primarily in type 2 diabetic adults (Blankenship JM, Granados K, & Braun B, 2014).

Therefore, the aim of this review was to express the importance of glycemic variability, its relationship with sedentary behavior and physical activity, and how a

single bout of exercise or repeated bouts of exercise, as well as exercise training influences glycemic variability in a variety of populations. Further, with more recent technological advancement of CGM to aid in assessment of glycemic health, glycemic variability has become an integral assessment for prevention and/or treatment in those at risk of or currently diagnosed with cardiometabolic diseases. Based on evidence provided in this review, practical implications for use of glycemic variability in assessment of glycemic health in relation to physical activity and exercise, will be provided.

METHODS

To address the overall study purpose, we searched the electronic databases PubMed, Google Scholar, Web of Science, BioMed Central, Cumulative Index of Nursing and Allied Health Literature (CINAHL), and SCOPUS. We limited the date range until December 2019 with a secondary search strategy included scanning bibliographies of the retrieved articles. Initial selection was based on title and abstract. The original article was obtained and subsequently determined whether the research study met the inclusion criteria established for the critical review. Articles were reviewed based on the criteria provided in Table 3.1. and 3.2. and extracted details included population, treatment, summary results of primary findings, and potential strength and limitations of each study.

Glycemic variability included only CGM-assessed glycemic variability. Glycemic variability has commonly been utilized interchangeably with glycemic excursions and glucose or glycemic control, which were therefore included in the search terms. Further, multiple brands and models of CGM devices exist that have been proven reliable and valid compared to venous blood glucose concentrations, including, but not limited to

Dexcom (San Diego, CA), FreeStyle (Abbott Laboratories; Chicago, IL), Guardian (Medtronic; Dublin, Ireland), and Eversense (Senseonics Holdings; Germantown, MD). Hence, it was ensured that the CGM devices utilized in the present review had been previously tested for accuracy in comparison to blood glucose concentrations.

Sedentary behaviors were included, which comprised of time spent sedentary and the classical definition of sedentary behavior, which has been expressed as any waking behavior characterized by an energy expenditure ≤1.5 metabolic equivalents (Tremblay MS, Aubert S, Saunders TJ, CarsonV, Latimer-Cheung AE, Chastin SFM, Altenberg TM, & Chinapaw MJM, 2017). Physical activity included outcomes measured by subjectively validated questionnaires or objectively utilizing accelerometry and/or pedometers. Exercise has been classically defined as structured physical activity performed with a purpose or goal (Caspersen CJ, Powell KE, & Christenson GM, 1985). Therefore, we also examined how a single bout of exercise or repeated bout of exercise, as well as exercise training, affects glycemic variability. When examining the influence of exercise, we included any modality or duration of submaximal and maximal bouts of exercise tests and defined the acute response as immediately post-exercise up to 48 hours post-exercise. Further, we included exercise interventions of any duration if they met the minimum guidelines established for being considered physically active by a reputable governing body, such as the Department of Health and Human Services, American Diabetes Association, American Heart Association, and/or the American College of Sports Medicine.

General Discussion

Importance of Glycemic Variability

Glycemic control often refers to hemoglobin A1c (HbA1c); however, glycemic control is often utilized interchangeably with glycemic variability. Yet, these measurements more so complement each other in addition to portraying similar concepts. As glycemic variability accounts for oscillations in glucose concentrations over a period of time, glycemic control has historically been comprised of HbA1c evaluation, which accounts for the average volume of glucose bound to the hemoglobin of circulating red blood cells over ~3 months (American Diabetes Association, 2020). Most type 1 and type 2 diabetic patients tend to have HbA1c measured every 3 months in hopes of determining whether their glycemic control targets have been obtained and/or maintained (Wei N, Zheng H, & Nathan DM, 2014). Impaired glycemic control assessed by HbA1c has a strong predictive value for diabetes onset and diabetic complications (Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, & Holman RR, 2000; Little RR, Rohlfing CL, Sacks DB, National Glycohemoglobin Standardization Program Steering Committee, 2011; Laiteerapong N, Ham SA, Gao Y, Moffet HH, Liu JT, Huang ES, & Karter AJ, 2019). Thus, glycemic control assessed as HbA1c has been established as a useful tool for a point-of-care opportunity between healthcare providers and patients for more timely treatments for optimizing glycemic management to deter the progression of diabetic complications.

However, HbA1c assessed glycemic control has its limitations as a sole measure of glycemic health, as the cut-point of HbA1c from the diagnostic point of view is still controversial (Sherwani SI, Khan HA, Ekhzaimy A,Masood A, & Sakharkar MK, 2016).

The HbA1c test is considered an indirect measure of average glycemia and uncertain variability is often unaccounted for as red blood cells may be affected by cell turnover, anemia, blood transfusions, and pregnancy (Jovanovič L, Savas H, Mehta M, Trujillo A, & Pettitt DJ, 2011; Beck RW, Connor CG, Mullen DM, Wesley DM, & Bergenstal RM, 2017). Additionally, population differences exist in HbA1c, in which ethnicity/race, sex, and age may influence reference HbA1c concentrations and potential diabetic-related complications (Cavagnolli G, Pimental AL, Freitas PAC, Gross JL, & Carmargo JL, 2017). Specifically, HbA1c has been observed to be greater in African Americans compared to Caucasians, males compared to females, as well as increases with age (Ma Q, Liu H, Xiang G, Shan W, & Xing W, 2016).

Recently, evaluation of HbA1c has been accompanied by further testing, to examine glucose concentrations in a non-clinical setting, i.e. 24-hour, diurnal, and nocturnal, in addition to traditional clinical examinations of glycemic health, such as fasting or in a glucose challenged state (Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, Bosi E, Buckingham B, Cefalu WT, Close KL, Cobelli C, Dassau E, DeVries JH, Donaghue KC, Dovc K, Doyle FJ, Garg S, Grunberger G, Heller S, Heinemann L, Hirsch IB, Hovorka R, Jia W, Kordonouri O, Kovatchev B, Kowalski A, Laffel L, Levine B, Mayorov A, Mathieu C, Murphy HR, Nimri R, Nørgaard K, Parkin CG, Renard E, Rodbard D, Saboo B, Schatz D, Stoner K, Urakami T, Weinzimer SA, & Phillip M, 2019). The relationship between glucose concentrations and HbA1c within an individual correlate over time (Chehregosha H, Khamesh ME, Malek M, Hosseinpanah F, & Hosseinpanah F, 2019). More specifically, the more time-point measurements to analyze mean glucose concentration utilizing CGM technology, the stronger the

correlation observed with HbA1c (Hirsch IB, Welsh JB, Calhoun P, Puhr S, Walker TC, & Price DA, 2019). Additionally, glycemic states, including euglycemia and hyperglycemia, have been previously reported to strongly correlate with HbA1c (Hirsch IB, Welsh JB, Calhoun P, Puhr S, Walker TC, & Price DA, 2019). Therefore, with the advent of new technology, CGM has evolved rapidly in both accuracy and affordability and allows for evaluation of free-living glucose concentrations and glycemic variability in addition to glycemic control established as HbA1c.

Glycemic Variability Measures

Currently, several measures of glycemic variability are utilized and range in sensitivity and specificity (Rodbard D 2018). These include measurements to establish intra-day and inter-day glycemic variability, with many designed for CGM technology (Suh S & Kim JH, 2015). Glycemic variability measurements include those commonly used for ease of analysis, such as the standard deviation (SD) of the mean glucose concentration and percentage coefficient of variation (%CV). Additionally, more sensitive analyses of glycemic variability have been adopted, including the mean amplitude of glycemic excursions (MAGE), continuous overlapping net glycemic action over an *n*-hour period (CONGA-*n*), and the mean of daily differences (MODD). In the following section, the most frequently used, but not all-inclusive, measurements of glycemic variability will be discussed, with strengths and limitations listed (Table 4.1.).

Prior to validation of more sensitive measures of glycemic variability, the SD of mean glucose concentration and %CV were utilized (Kovatchev B, Anderson S, Heinemann, & Clark W, 2008). Statistically, SD and %CV are often thought of as the best measurement for variation since they are based on all measurements in a distribution

(Altman DG & Bland JM, 2005). Additionally, %CV is considered potentially more inclusive and sensitive compared to SD alone as it incorporates both the mean and SD of the distribution (Kovatchev B, Anderson S, Heinemann, & Clark W, 2008). Even though these measurements include all glucose concentrations, there exists an inherent limitation that does not account for individual physiological differences or differences in lifestyle factors, such as physical activity, exercise, and dietary consumption, which should be considered. Even so, a large body of evidence supports that a direct linear relationship exists between SD and %CV for a large series of CGM data with more sensitive measures of glycemic variability (Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, & ADAG Study Group, 2008; Kuenen JC, Borg R, Kuik DJ, Zheng H, Schoenfeld D, Diamant M, Nathan DM, Heine RJ, & ADAG Study Group, 2011).

One of the initial measurements of intra-day glycemic variability was MAGE (Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, & Taylor WF, 1970). MAGE is calculated by taking the arithmetic mean of the blood glucose increases or decreases (from blood glucose nadirs to peaks or vice versa) when both ascending and descending segments exceeded the value of one SD of the mean blood glucose for the same 24-hour period (Service FJ & Nelson RL, 1980). MAGE does not account for time spent in euglycemia, or periods of low-level hypo- or hyper-glycemia (Service FJ, O'Brien PC, & Rizza RA, 1987). This creates ambiguity as to where glycemic excursions occur, potentially limiting the ability to measure the magnitude of glycemic excursions (Rodbard D 2009). However, MAGE provides insight into the extent that glycemic excursions occur, specifically accounting for postprandial hyperglycemia and fasted-state hypoglycemia.

Further consideration for time-dependent glycemic variability, which utilizes the continuous nature of CGM, was established with other measures. CONGA-*n* was formulated to account for each observation after the first *n* hours of observations (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005). Further, the difference between the current observation and the observation *n* hours previous is calculated with CONGA-*n* defined as the SD of the differences. (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005). CONGA-*n* can be calculated from 1 to 24-hour time intervals; yet, has been utilized most frequently for 1- to 4-hour time intervals to account for time between certain activities throughout the day, with accuracy decreasing beyond the 4-hour time interval (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005; Kuenen JC, Borg R, Kuik DJ, Zheng H, Schoenfeld D, Diamant M, Nathan DM, Heine RJ, & ADAG Study Group, 2011; Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, & ADAG Study Group, 2008). For example, CONGA-1 was initially created as a measurement of glycemic variability to account for glycemic excursions throughout a 24-hour period, while CONGA-2 accounts for time between snacks, and CONGA-4 as the variability observed between standard meals, which include breakfast, lunch, and dinner (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005). CONGA-*n* continues to be a more promising measure of intra-day glycemic variability and provides practitioners with more flexibility in the evaluation of intra-day glycemic variability (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005).

In addition to intra-day glycemic variability, use of CGM allows for analysis of inter-day glycemic variability. The MODD measure of inter-day glycemic variability was

designed as a meticulous protocol occurring over two consecutive days during in-house clinical testing (Service FJ & Nelson RL, 1980; Service FJ, O'Brien PC, & Rizza RA, 1987). MODD is traditionally calculated as the absolute value of the difference between glucose concentrations measured at the same time over a 24-hour period on two consecutive days (Service FJ & Nelson RL, 1980; Service FJ, O'Brien PC, & Rizza RA, 1987). MODD may also be calculated as a continuous time series over an extended period (>2 days), which has been shown to strongly correlate with MODD over the initial two consecutive days under standardized conditions (Rodbard D 2009).

With the advancement and incorporation of CGM technology, the ability to design and implement more sensitive and time specific measures of glycemic variability have become a viable option when delineating glycemic health. In addition to the SD and %CV of measured glucose concentrations over an extended period, the ability to measure inter- and intra-day variability has been further incorporated using MAGE, CONGA-*n*, and MODD. Even though multiple measures of glycemic variability have been developed, most of the research has been performed in those with impaired glucose tolerance or type 1 and type 2 diabetes. Thus, inclusion of these more sensitive measures should be considered when evaluating the variation in glucose concentrations in other populations at an increased risk for the development of cardiometabolic disorders, such as sedentary healthy weight, and/or overweight or obese adults.

Relationship with Sedentary Behavior and Physical Activity

Decreasing sedentary time and increasing physical activity of any intensity is widely accepted to be beneficial for overall and cardiometabolic health (Young DR, Hivert MF, Alhassan S, Camhi SM, Ferguson JF, Katzmarzyk PT, Lewis CE, Owen N,

Perry CK, & Yong CM, 2016; Katzmarzyk PT, Powell KE, Jakicic JM, Troiano RP, Piercy K, & Tennant B, 2019 2017). Further, sedentary time and physical activity are independently associated with elevated fasting glucose concentrations, impaired glucose metabolism, and glucose tolerance in non-diabetic and diabetic adults (Høstmark AT, Ekeland GS, Beckstrøm AC, & Meen HD, 2006; Helmerhorst HJ, Wijndaele K, Brage S, Wareham NJ, & Ekelund U, 2009; Dunstan DW, Barr EL, Healy GN, Salmon J, Shaw JE, Balkau B, & Owen N, 2010; Ford ES, Zhao G, & Li C, 2010; Thorp AA, Healy GN, Owen N, Slamon J, Ball K, Shaw JE, & Dunstan DW, 2010; Veerman JL, Healy GN, Cobiac LJ, Vos T, Winkler EA, Owen N, & Dunstan DW, 2012; Lahjibi E, Heude B, Dekker JM, Højlund K, Laville M, Nolan Jn & Balkau B, 2013; Nygaard H, Grindaker E, Rønnestad BR, Holmboe-Ottesen G, & Høstmark AT, 2017). In adults diagnosed with type 2 diabetes, increased time spent sedentary has previously been shown to predict significant increases in time spent in hyperglycemia (Fritchi C, Park, H, Richardson, A, Park C, Collins EG, Mermelstein R, Riesche L, & Quinn L, 2016). These findings highlight the relationship between time spent sedentary and performing physical activity with glucose concentrations and glucose tolerance. However, there remains limited evidence of a relationship between sedentary behavior and/or physical activity with glycemic variability (Table 4.2.).

A prospective repeated measures designed study that included 35 young adults (18-35 years of age) diagnosed with type 1 diabetes found no relationship between total physical activity minutes and the SD of the 24-hour mean glucose concentration (β estimate=2.2031, p=0.127) (Martyn-Nemeth P, Quinn L, Penckofer S, Park C, Hofer V, & Burke L, 2017). Additionally, a study performed by Paing et al. examined the

associations between objectively measured sedentary time with 24-hour glycemic control in 37 type 2 diabetic adults (>18 years of age) (Paing AC, McMillan KA, Kirk AF, Collier A, Hewitt A, & Chastin SFM, 2018). This study found that time spent sedentary was positively associated with poorer CGM-assessed glycemic control, and in those who spent more time sedentary there was a noted reduction for time spent in euglycemia (β estimate=-0.44, p=0.04) and a trending relationship with increased time spent hyperglycemic (β estimate=0.36, p=0.08). However, breaks in sedentary time was positively associated with time spent in euglycemia (β estimate=0.38, p=0.04). Further, a cross-sectional study of non-diabetic and diabetic adults participating in the A Estrada Glycation and Inflammation Study had a subsample of 511 adults (12% diagnosed type 1 and 2 diabetic) complete the International Physical Activity Questionnaire and had valid CGM monitoring data for analysis (Gude F, Díaz-Vidal P, Rúa-Pérez C, Alonso-Sampedro M, Fernández-Merino C, Rey-García J, Cadarso-Suárez C, Pazos-Couselo M, García-López JM, & Gonzalez-Quintela A, 2017). This study found no relationship between physical activity status with glycemic variability measures, including the SD of 24-hour mean glucose concentration, MAGE, and CONGA-1 in the non-diabetic adult population ($p \geq 0.264$ for all), but did not report findings in the diabetic population.

These studies highlight the potential importance of sedentary behavior and physical activity with glycemic control and glycemic variability assessed by CGM in a variety of populations. Even though most previous findings are limited to glycemic control assessed as HbA1c, these studies provide insight into how CGM may be incorporated as a measure of glycemic control and glycemic variability in addition to traditional clinical measures. However, these studies did not definitively distinguish

differences between non-diabetic and diabetic adults or utilized subjective rather than objective assessments of sedentary behavior and physical activity. Hence, evidence suggests that a potential and impactful relationship between sedentary behavior and physical activity with CGM-assessed glycemic control and glycemic variability exists in adults with a compromised metabolic profile, such as type 2 diabetes. However, when examining non-diabetic adults and/or adults which may utilize other lifestyle management techniques or medications to influence their glycemic health, such as type 1 diabetics, further consideration for other lifestyle factors, including free-living dietary patterns or structured physical activity (e.g. exercise), may be pertinent when evaluating glycemic health with CGM technology.

Influence of Single Bout or Repeated Bouts of Exercise on Glycemic Variability

Exercise has been shown to beneficially influence insulin resistance and glucose tolerance, and is commonly utilized as a treatment for both non-insulin and insulin-dependent type 2 diabetes (Joslin EP, Root HF, White P, & Marble A, 1935; Goodyear LJ & Kahn BB, 1998). Even a single bout of exercise can have a beneficial impact on glucose homeostasis, whole-body glucose disposal, and increase glucose uptake into skeletal muscle (Pruett EDR & Oseid S, 1970; Bogardus C, Thuillex P, Ravussin E, Vasquez B, Narimiga M, & Azhar S, 1983; Richter EA, Mikines KJ, Galbo H, & Kiens B, 1989; Wang X, Patterson BW, Smith GI, Kampelman J, Reeds DN, Sullivan SA, & Mittendorfer B, 2013). Further, a single bout of exercise may have persistent effects on glucose uptake and insulin sensitivity for several hours after completion of exercise (Devlin JT & Horton ES, 1985; Devlin JT, Hirshman MF, Horton ES, & Horton ED, 1987; Mikines KJ, Sonne B, Farrell PA, Tronier B, & Galbo H, 1988). Yet, few studies

have examined the impact of a single bout of exercise or repeated bouts of exercise on CGM-assessed glycemic control and glycemic variability.

Previous studies examining the influence of a single bout of exercise or repeated bouts of exercise on CGM-assessed glycemic control and glycemic variability in non-diabetic, healthy weight and overweight or obese adults have found comparable and consistent results (Table 4.3.) (Little JP, Jung ME, Wright AE, Wright W, & Manders JF, 2014; Parker L, Shaw CS, Banting L, Levinger I, Hill KM, McAinch AJ, & Stepto NK, 2017; Figueira FR, Umpierre D, Bock PM, Waclawovsky G, Guerra AP, Donelli A, Andrades M, Casali KR, & Schaan BD, 2019). Figueira et al., found that a single bout of moderate-intensity aerobic exercise and eccentric resistance exercise had comparable decreases in glycemic variability compared to pre-exercise control period 15 young healthy adults (Figueira FR, Umpierre D, Bock PM, Waclawovsky G, Guerra AP, Donelli A, Andrades M, Casali KR, & Schaan BD, 2019). Additionally, Little et al., found that postprandial glycemic excursions decreased following high-intensity interval training (HIIT) compared to continuous moderate-intensity exercise and control conditions, in 10 inactive overweight adults (Little JP, Jung ME, Wright AE, Wright W, & Manders JF, 2014). Lastly, Parker et al., found that 24-hour average glucose concentration and hyperglycemic excursions decreased following either low-volume high-intensity interval exercise or continuous moderate-intensity exercise in 27 overweight or obese normoglycemic adults (Parker L, Shaw CS, Banting L, Levinger I, Hill KM, McAinch AJ, & Stepto NK, 2017) These studies highlight that a single bout of moderate-intensity aerobic exercise, HITT, and eccentric resistance exercise improve glycemic control and glycemic variability. Further, glycemic control and glycemic

variability were measured in a variety of ways, including 24-hour mean glucose concentration, SD and %CV of the 24-hour mean glucose concentration, and postprandial glycemic excursions. Therefore, in normal weight and overweight or obese adults, a single bout of exercise or single session of repeated bouts of exercise, regardless of modality or duration, potentially provides beneficial and lasting effects on 24-hour glycemic control and glycemic variability.

Even though observable effects of a single bout or repeated bouts of exercise on glycemic control and glycemic variability in adults have been found, the effects on those with diagnosed glycemic dysfunction may be of greater importance. The ability to improve glycemic control and glycemic variability with exercise in those who rely on lifestyle self-management techniques to control their glucose levels, such as adults diagnosed with type 1 diabetes, potentially has profound implications. Previous research under free-living or clinic-based conditions have found that increasing structured physical activity, or exercise, may have differential effects on glycemic control and glycemic variability, depending on type of exercise, in type 1 diabetic adults (Manohar C, Levine JA, Nandy DK, Saad A, Man CD, McCrady-Spitzer SK, Basu R, Cobelli C, Carter RE, Basu A, & Kudva YC, 2012; van Dijk J-W, Eijsvogels TM, Nyakayiru J, Schreuder THA, Hopman MT, Thijssen DH, & van Loon LJC, 2016). In turn, there are mixed findings, as one study noted differential changes in free-living glycemic control or glycemic variability following repeated bouts of exercise, while clinic-based exercise influenced glycemic control and glycemic variability in type 1 diabetic adults (Manohar C, Levine JA, Nandy DK, Saad A, Man CD, McCrady-Spitzer SK, Basu R, Cobelli C, Carter RE, Basu A, & Kudva YC, 2012; van Dijk J-W, Eijsvogels TM, Nyakayiru J,

Schreuder THA, Hopman MT, Thijssen DH, & van Loon LJC, 2016). van Dijk et al., found that glycemic variability increased during a 4-day walking event compared to habitual physical activity control period in 10 type 1 diabetic adults; however, noted that insulin dosage decreased and total energy intake, specifically calories composed of carbohydrates, increased during the 4-day walking event compared to habitual physical activity (van Dijk J-W, Eijsvogels TM, Nyakayiru J, Schreuder THA, Hopman MT, Thijssen DH, & van Loon LJC, 2016). Yet, under controlled clinic-based assessment, Manohar et al., found that intermittent bouts of low-volume and low-intensity walking decreased post-meal glucose area under the curve and glycemic excursions compared to inactivity in 12 healthy control and 12 type 1 diabetic adults (Manohar C, Levine JA, Nandy DK, Saad A, Man CD, McCrady-Spitzer SK, Basu R, Cobelli C, Carter RE, Basu A, & Kudva YC, 2012). Therefore, alterations in insulin dosage and energy intake in a free-living condition may limit the impact of increased energy expenditure and exercise on glycemic control and glycemic variability in type 1 diabetics that may not be accounted for under clinic-based assessment. Additionally, free-living assessment may be more pertinent than clinic-based assessment as adults diagnosed with type 1 diabetes rely more heavily on lifestyle management techniques compared to non-diabetic and non-insulin-treated type 2 diabetic adults to control their blood glucose concentrations.

As previously highlighted, a single bout or repeated bouts of exercise have been shown to improve overall glycemic health, including glucose concentrations, glycemic excursions, and glycemic variability in healthy weight, overweight or obese adults, as well as adults diagnosed with type 1 diabetes. However, the most prevalent population which could benefit from the effects of exercise on glycemic control and glycemic

variability are adults diagnosed with type 2 diabetes (Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, & Braun B, 2010). Previous evidence in adults diagnosed with impaired glucose tolerance and type 2 diabetes, whether insulin-treated or non-insulin treated, suggests improvements in glycemic control and glycemic variability regardless of modality of exercise (Praet SF, Manders RJ, Lieverse AG, Kuipers H, Stehouwer CD, Keizer HA, & van Loon LJ, 2006; Figueira FR, Umpierre D, Casali KR, Tetelbom PS, Henn NT, Ribeiro JP, & Schaan BD, 2013; van Dijk J-W, Manders RJF, Canfora EE, Van Mechelen W, Hartgens F, Stehouwer CDA, & van Loon LJC, 2013; Farabi SS, Carley DW, Smith D, & Quinn L, 2015). Farabi et al., found that in adults with impaired glucose tolerance or type 2 diabetes, a single 30-minute bout or moderate-intensity exercise significantly and differentially decreased diurnal CONGA-1 when compared to pre-exercise values and change values compared to non-diabetic adults (Farabi SS, Carley DW, Smith D, & Quinn L, 2015). van Dijk et al., noted decreased CONGA-1, CONGA-2, and CONGA-4 following a single bout of 45-60 minutes moderate-intensity aerobic exercise in 60 non-insulin and insulin-treated type 2 diabetic adults (van Dijk J-W, Manders RJF, Canfora EE, Van Mechelen W, Hartgens F, Stehouwer CDA, & van Loon LJC, 2013). Additionally, Praet et al., observed decreases in hyperglycemic excursions following a single session of low-weight/high-volume resistance exercise, which incorporated upper- and lower-body, as well as abdominal exercise in 11 insulin-treated type 2 diabetic adults (Praet SF, Manders RJ, Lieverse AG, Kuipers H, Stehouwer CD, Keizer HA, & van Loon LJ, 2006). However, Figueira et al., found that despite comparable decreases in glucose concentrations following either a single 40-minute bout of continuous moderate-intensity

aerobic exercise or combined aerobic plus whole-body resistance exercise, there were no changes in 24-hour glucose variance or %CV following either exercise modality in 14 type 2 diabetic adults (Figueira FR, Umpierre D, Casali KR, Tetelbom PS, Henn NT, Ribeiro JP, & Schaan BD, 2013). Therefore, findings from these studies varied based on glycemic control and glycemic variability evaluation method, which suggested changes in only intra-day, but not inter-day, glycemic control and glycemic variability occurred following a single bout or repeated bouts of exercise. Additionally, those studies that presented findings extending into the subsequent day following exercise session found that there were no persistent changes in glycemic control or glycemic variability beyond the initial 24-hour period (Praet SF, Manders RJ, Lieverse AG, Kuipers H, Stehouwer CD, Keizer HA, & van Loon LJ, 2006; Figueira FR, Umpierre D, Casali KR, Tetelbom PS, Henn NT, Ribeiro JP, & Schaan BD, 2013). These studies highlight the importance of a single bout or repeated bouts of exercise, regardless of modality, on short-term glycemic control and glycemic variability in type 2 diabetic adults, which would potentially be the most impacted group to focus on when attempting to improve glycemic profiles through targeted exercise prescription. Yet, the findings indicate that glycemic control and glycemic variability may not persist past the subsequent 24-hour monitoring period, suggesting that continuous participation in exercise programs may be needed to implant long-term changes in glycemic control and glycemic variability in type 2 diabetic adults.

Influence of Exercise Training on Glycemic Variability

In the previous section, a single bout of exercise or repeated bouts of exercise were shown to improve glycemic profiles for varying measurement of glucose

metabolism, including glycemic excursions and glycemic variability that occur throughout the day. Even though a single bout or repeated bouts of exercise are beneficial for glycemic health, additional long-term adaptations and enduring improvements in glucose metabolism occur due to extended exercise participation. Improvements in glycemic control due to exercise training are primarily explained by an increase in whole-body glucose disposal and insulin sensitivity (Scheider SH, Amorosa LF, Khachadurian AK, & Ruderman NB, 1984). Yet, these adaptations are reversible, and the effects of detraining begin within 5-10 days after cessation of exercise (Mikines KJ, Sonne B, Farrell PA, Tronier B, & Galbo H, 1988). Therefore, general involvement and, more importantly, continuation of regular exercise participation are necessary to impact long-term glycemic health.

Epidemiological studies have determined that regular exercise participation training can reduce the risk for developing non-insulin-dependent diabetes (Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, Rosner B, Hennekens CH, & Speizer FE, 1991; Manson JE, Nathan DM, Krolewski AS, Stampfer MJ, Willett WC, & Hennekens CH, 1992; Helmrich SP, Ragland DR, Leung RW, Paffenbarger RS, 1991). Furthermore, a brief review and preliminary evidence published by Holloszy et al., suggested that a decline in glucose tolerance and insulin sensitivity can be prevented by performing regular exercise (Holloszy JO, Schultz J, Kusnierkiewicz J, Hagberg JM, & Ehsani AA, 1986). They also provided evidence that prolonged and frequent exercise can normalize glucose tolerance by decreasing insulin resistance in some individuals with impaired glucose tolerance. However, to date, few published studies have evaluated changes in glycemic control and glycemic variability following exercise training as

opposed to general participation in exercise. Furthermore, these studies examining the effects of exercise training on glycemic variability have primarily included adults diagnosed with type 2 diabetes. Therefore, exercise training interventions of a week or greater are included in this section to aid the inclusion of pertinent literature, as there are limited published findings (Table 4.4.).

Despite the limited number of exercise training studies on this topic, the evidence remains consistent with previous literature observing the influence of a single bout of exercise or repeated bouts of exercise in non-diabetic, as well as type 1 and type 2 diabetic adults. Mikus et al., suggested that even a week (7 days) of moderate-intensity aerobic exercise training (~60 minutes per exercise session) positively influences glycemic control and glycemic variability in 13 type 2 diabetic adults, in the absence of alterations to traditional clinic-based assessment of glycemic health, including fasting and OGTT glucose concentrations (Mikus CR, Oberlin DJ, Libla J, Boyle LJ, & Thyfault JP, 2012). Additionally, studies which incorporated extended exercise programs have found that alterations in glycemic control and glycemic variability may be intensity dependent rather than due to general participation in exercise. These studies found that high-intensity interval exercise, including HIIT and interval walking, as opposed to low-intensity continuous exercise may be more beneficial for glycemic control and glycemic variability in type 2 diabetic adults (Karstoft K, Winding K, Knudsen SH, Nielsen JS, Thomsen C, Pedersen BK, & Solomon TPJ, 2013; Francois ME, Durrer C, Pistawka KJ, Halperin FA, Chang C, & Little JP, 2017). Karstoft et al., found that CGM-assessed glycemic control group worsened in a non-exercise control group (n=8), while glycemic control improved following 4 months of 60 minutes per day of interval walking (n=12)

with no changes following 4 months of 60 minutes per day of continuous walking (n=12). Further, Francois et al., found that 12 weeks of prescribed HIIT, which met exercise prescription criteria established by the American Diabetes Association and the American College of Sports Medicine, decreased glycemic variability measured as SD of 24-hour mean glucose concentration and MAGE compared to pre-exercise training values in 53 type 2 diabetic adults (Francois ME, Durrer C, Pistawka KJ, Halperin FA, Chang C, & Little JP, 2017).

Therefore, these findings provide evidence that regular exercise participation, specifically high-intensity or interval exercise, has the potential to improve glycemic control and glycemic variability in type 2 diabetic adults. Additionally, the use of supervised exercise, including aerobic and resistance, or free-living walking interventions, support feasibility of implementation of exercise of varying modalities as a therapeutic for improvements in glycemic control and glycemic variability in adults diagnosed with type 2 diabetes. Thus, this allows for further consideration to be made for type 1 diabetic adults and overweight or obese, but otherwise healthy adults, to aid in the implementation of preventive approaches to decrements in metabolic health as opposed to treatment in those metabolically compromised.

Strengths and Limitations

Overall, there is a lack of well-documented studies that examine the relationship between sedentary time and physical activity with CGM-assessed glycemic control and glycemic variability, and even less information regarding the effect of a single bout or repeated bouts of exercise and chronic exercise training on these outcomes of interest. This review aimed to be among the first to critically examine the current literature

regarding sedentary time and physical activity with glycemic variability and compile evidence to delineate the gaps in the literature that need to be addressed. This review also reported findings of a single bout of exercise or repeated bouts of exercise and exercise training and their respective effects on glycemic control and glycemic variability. Inclusion of multiple source databases, including PubMed, Google Scholar, Web of Science, BioMed Central, CINAHL, and SCOPUS up until December 2019 was another strength. Additionally, a variety of search terms for each outcome variable of interest were utilized.

There are several limitations to this study. The first was incorporation of subjectively assessed, in addition to objectively assessed, sedentary time and physical activity, as subjectively assessed sedentary time and physical activity often lack not only validity and reliability, but also reproducibility (Prince SA, Adamo KB, Hamel ME, Hardt J, Gorber SC, & Tremblay M, 2008). Additionally, primarily compiling articles utilizing CGM assessed glycemic variability is another limitation, as additional measures of glycemic control and glycemic variability exist. Yet, the accuracy of CGM measurements have been validated with clinical measures, such as venous blood sampling and allow for assessment of free-living glycemic control and glycemic variability (Akintola AA, Noordam R, Jansen SW, de Craen AJ, Ballieux BE, Cobbaert CM, Mooijaart SP, Pijl H, Westendorp RG, & van Heemst D, 2015). Lastly, the inclusion of a variety of populations is a limitation but allows for meaningful observations as to how sedentary time and physical activity relate to glycemic control and glycemic variability, as well as provides greater insight into the influence of a single bout or

repeated bouts of exercise, or exercise training on glycemic control on glycemic variability.

With these strengths and limitations in mind, future studies should potentially focus on increasing both exercise and non-exercise related physical activity, as well as selecting and employing an appropriate volume of exercise depending on the population in question. Further, other exercise characteristics, such as frequency and intensity may be utilized to tailor exercise interventions to match individual preferences and abilities in addition to targeting beneficial changes in glycemic control and glycemic variability. Therefore, practitioners should encourage participants to decrease sedentary behavior and increase physical activity throughout the day, as well as to implement exercise, regardless of modality, into their everyday life to improve cardiometabolic health outcomes, specifically long-term glycemic control and glycemic variability.

CONCLUSION

Although physical activity and exercise are important treatment strategies to improve long-term glycemic control, the relationship between sedentary time and physical activity, and the impact of exercise on free-living glycemic control and glycemic variability remains largely unexplored. The introduction and advancement of CGM technology enables researchers and practitioners to assess, as well as evaluate how lifestyle factors impact glycemic health in a free-living environment. As evidenced in this review, the current literature utilizing CGM technology has demonstrated that potential relationships exist between glycemic control and glycemic variability with habitual sedentary time and physical activity. Further, this review found that a single bout of exercise or repeated bouts of exercise, as well as exercise training, may beneficially

impact glycemic control and glycemic variability immediately following exercise, specifically in adults diagnosed with type 2 diabetes.

Table 4.1. Frequently used measurements of glycemic variability

Glycemic variability measurement	Calculation definition	Strengths	Limitations	Author (publication date)
Standard deviation (SD)	SD of all glucose concentrations in a distribution.	Simple, classical statistical method.	Does not account for skewed distributions or outliers.	Pernick NL & Rodbard D (1986); Rodbard D (1988); Hirsch IB (2005); Rodbard D (2007)
Percentage coefficient of variation (%CV)	(SD÷mean)×100 SD and mean value of all glucose concentrations in a distribution.	Simple, classical statistical method. Incorporates both the SD and mean value of a distribution.	Does not account for skewed distributions or outliers.	Pernick NL & Rodbard D (1986); Rodbard D (1988); Hirsch IB (2005); Rodbard D (2007)
Mean amplitude of glycemic excursions (MAGE)	Average amplitude of upstrokes or downstrokes with a magnitude >1 SD above the mean value for all glucose concentrations.	Account for physiological fluctuations due to events throughout the day. e.g. meals, exercise	Less efficient to calculate than SD, while providing similar outcomes.	Service FJ et al. (1970); Service FJ & Nelson RL (1980); Service et al. (1987)
Continuous overlapping net glycemic action over n-hour (CONGA-*n*)	The SD of the difference between two glucose concentrations obtained exactly *n* hours apart.	Potential to address a variety of clinical questions. CONGA-1 to CONGA-4 valid and reliable when accounting for corresponding times	Validity and reliability decrease once time frame >4 hours in a controlled setting.	McDonnell CM et al. (2005); Kuenen JC et al. (2008); Nathan DM et al. (2008)

		between different activities.		
Mean of daily differences (MODD)	Mean of absolute differences between glucose values obtained at the same time of day on two consecutive days under standardized conditions. Mean of absolute differences in glucose values over >2 days between any value and the value exactly 24 hours later.	Describes between-day variability. Ability to permit use of data from >2 unstructured days.	Originally defined for two consecutive days assuming similar meals, activities, and therapy on both days.	Service FJ & Nelson RL (1980); Service et al. (1987)

Table 4.1. provides frequently used measurements of glycemic variability, which includes calculation definition of each glycemic variability measurement with further consideration for strengths and limitations of each glycemic variability measurement.

Table 4.2. Relationship between sedentary behavior and physical activity with glycemic control and glycemic variability

Author (publication date)	Study design Number of participants	Primary findings	Conclusion	Strengths and limitations
Non-diabetic				
Gude F et al. (2017)	Cross-sectional; N=622	No relationship found between physical activity status with any glycemic variability indices in non-diabetic adults.	Physical activity status may not relate to glycemic variability indices in non-diabetic adults.	Strengths: Large diverse sample of adults Limitations: Cross-sectional design; subjective assessment of physical activity status
Type 1 diabetes				
Martyn-Nemeth P et al. (2017)	Prospective repeated-measures design; N=35	Total physical activity minutes did not relate to glycemic variability assessed as the SD of the 24-hour mean glucose concentration.	Increases in total physical activity performed throughout the day may not relate to lower glycemic variability in type 1 diabetic adults.	Strengths: Actigraphy-assessed physical activity Limitations: Small sample size; physical activity was not a primary outcome of this study
Type 2 diabetes				
Paing AC et al. (2018)	Cross-sectional; N=37	Time spent in euglycemia negatively associated with sedentary	Decreasing sedentary time, breaking up sedentary time, or a combination	Strengths: Actigraphy-assessed sedentary time Limitations:

	time, but positively associated with breaks in sedentary time.	of these sedentary behaviors potentially influence time spent in euglycemia and glycemic control without impacting hypo- or hyperglycemia in type 2 diabetic adults.	Cross-sectional design; small sample size

Table 4.2. presents studies that provided information regarding the association between sedentary time and physical activity with glycemic control and glycemic variability in non-diabetic, as well as type 1 and type 2 diabetic adults. The table includes: 1) author information; 2) study design; 3) findings related to the association between sedentary time and physical activity with glycemic control and glycemic variability; 4) conclusions derived from the findings between the relationship between sedentary time and physical activity with glycemic control and glycemic variability; 5) strength and limitations of each study.

Table 4.3. Influence of a single bout of exercise or repeated bouts of exercise on glycemic control and glycemic variability

Author (publication date)	Study design	Primary findings	Conclusion	Strengths and limitations
Non-diabetic				
Figueira FR et al. (2019)	Randomized crossover trial design; N=15 Two experimental sessions; aerobic cycle ergometry; eccentric resistance exercise	Glucose variance and glucose %CV and SD were lower at 12-18 hours post-exercise compared to pre-exercise in following both the aerobic bout and eccentric bouts of exercise.	Acute session of aerobic and eccentric exercise promotes comparable reductions in glycemic variability non-trained healthy adults.	Strengths: Controlled laboratory setting Limitations: Small sample size; exercise was of moderate to high intensity over an extended period
Little JP et al. (2014)	Randomized counterbalance trial design; N=10 Two 3-day experimental exercise testing periods; continuous moderate-intensity (CMI) exercise; high-intensity interval training (HIIT)	Absolute PPG spike following standardized breakfast and dinner were significantly lower following HIIT exercise compared to no-exercise control and following CMI exercise.	A single session of HIIT exercise improved overall postprandial glycemia in overweight or obese adults during the 24 h following a single training session in overweight or obese adults.	Strengths: Controlled laboratory setting. Limitations: Small sample size; inclusion of adults with impaired fating glucose
Parker L et al. (2017)	Randomized clinical trial; N=27 4-day experimental design; low volume high-intensity interval	24-hour post-exercise period following LV-HIIE, mean glucose and peak glucose concentration, and area under the curve were	LV-HIIE improves glycemic control to a similar extent as CMIE in overweight and obese adults.	Strengths: Controlled laboratory setting Limitations: Small sample size; participants

	exercise (LV-HIIE); continuous moderate-intensity exercise (CMIE)	lower compared to pre-exercise control and comparable to CMIE. Percent of time spent hyperglycemic was lower in LV-HIIE compared to pre-exercise control and comparable to following CMIE.	LV-HIIE may be a more effective exercise modality for incorporation into exercise programs designed to improve glycemic control in overweight or obese adults.	were not blinded to real-time CGM readings
Type 1 diabetes				
van Dijk JW et al. (2016)	Observational during world's largest walking event: Nijegen Four Day Marches; N=10 40-50 km walked per day over 4 days	CONGA-1 and CONGA-2 measures of glycemic variability were greater during the 4-day walking event compared to the habitual physical activity control day.	Prolonged continuous walking compared to habitual physical activity increased glycemic variability measures in type 1 diabetics.	Strengths: Examined a prolonged exposure to increase in physical activity Limitations: Small sample size; non-standard exercise modality
Manohar C et al. (2012)	Center-based clinical trial; N=24 Increase daily energy expenditure 3-fold from measured basal metabolic rate over three monitored days	In healthy controls %CV was lower following meals with physical activity compared to meals without physical activity; however, these findings were not observed in type 1 diabetics. Post-meal glycemic excursions were	Performing low-intensity physical activity after meals, such as taking a short walk, potentially benefit healthy and type 1 diabetics by lowering postprandial glucose excursions.	Strengths: Age- and sex-matched healthy controls and type 1 diabetics; controlled laboratory setting Limitations: Small sample size; type 1 diabetics received insulin boluses

		observed to be lower in controls and type 1 diabetics following meals with physical activity.		prior to their meals
Type 2 diabetes				
Farabi SS et al. (2015)	Center-based randomized clinical cross-over trial; N=37 Two 3-day experimental trials both in morning; sedentary for 30 minutes; 30-minute exercise session	Daytime CONGA-1 significantly decreased following exercise in segment 2 compared to sedentary trial in the type 2 diabetes/impaired glucose tolerance group.	A single bout of early morning moderate-intensity exercise reduced daytime glycemic variability in type 2 diabetic and/or impaired glucose tolerant obese adults.	Strengths: Controlled laboratory setting Limitations: Inclusion of adults with impaired glucose tolerance in the same group
van Dijk JW et al. (2013)	Randomized crossover trial; total N=60; non-insulin treated N=37; insulin treated=23 Two 3-day intervention periods separated by a week; sedentary protocol; 45-60 minutes of continuous cycling	Overall average 24-hour mean glucose concertation, time spent hyperglycemic, and CONGA-1, CONGA-2, and CONGA-4 measures of glycemic variability were all lower following a single bout of exercise.	A single bout of moderate-intensity exercise reduces hyperglycemia and glycemic variability throughout the subsequent day following exercise in non-insulin dependent and insulin dependent type 2 diabetic adults.	Strengths: Use of CGM; inclusion of insulin and non-insulin treated type 2 diabetics Limitations: Only inclusion of males
Praet SF et al. (2006)	Intervention-based clinical trial; N=11	Time spent hyperglycemic was significantly	As ingle bout of exercise reduces the	Strengths: Implementation of resistance

	Resistance exercise and aerobic exercise	lower during the subsequent 24 hours following exercise compared to 24 hours prior to exercise. No measures of glycemic variability differed between the 24-hour period pre- and post-exercise.	prevalence of hyperglycemia in long-standing, insulin-treated, type 2 diabetic male adults.	and aerobic exercise Limitations: Small sample size; large inter-subject variability
Figueria FR et al. (2013)	Randomized crossover design performed 7 days apart; N=14 Aerobic exercise; aerobic plus resistance exercise	24-hour mean glucose concentration comparably decreased in both groups post-exercise compared to pre-exercise. Larger sustained decrease in glucose concentrations in the aerobic plus resistance group compared to the aerobic only group were observed in the first 6-hours post-exercise. Changes in glycemic variability were noted in the aerobic plus resistance training group only when non-conventional symbolic	Both aerobic only and aerobic plus resistance exercise modalities decreased glucose levels over a short period of time following cessation of exercise. Conventional analyses of glycemic variability may lack sensitivity to account for minor oscillations in glucose concentrations observed using non-conventional analyses.	Strengths: Implementation of resistance and aerobic exercise; use of conventional and non-conventional methods Limitations: Small sample size; no resistance exercise only group

	analysis was applied.

Table 4.3. presents studies that provided information regarding the influence of a single bout of exercise or following repeated bouts of exercise on glycemic control and glycemic variability in non-diabetic, as well as type 1 and type 2 diabetic adults. The table includes: 1) author information; 2) study design; 3) findings related to the alterations in glycemic control and glycemic variability; 4) conclusions derived from the findings on changes in glycemic control and glycemic variability; 5) strength and limitations of each study.

Table 4.4. Influence of exercise training on glycemic control and glycemic variability

Author (publication date)	Study design	Primary findings	Conclusion	Strengths and limitations
Type 2 diabetes				
Mikus CR et al. (2012)	Clinical controlled trial; N=13 7-day moderate-intensity continuous aerobic exercise training program	Maximum glucose concentration, differences between maximum and minimum glucose concentration, and number of glucose excursions all decreased following over the final 3 days of the 7-day exercise training compared to 3 days of habitual daily activity.	7 days of aerobic exercise training reduces PPG and glycemic control in free-living individuals with type 2 diabetes but does not influence responses to the laboratory based OGTT	Strengths: Controlled exercise setting Limitations: Small sample size; volume of exercise performed was above recommended guidelines
Kartstoft K et al. (2013)	Randomized clinical trial performed over 4 months (16 weeks); total N=32; control group N=8; continuous-walking group N=12; interval-walking group N=12	24-hour mean, and minimum glucose concentrations increased in the control group, while 24-hour mean, and maximum glucose concentrations decreased in the interval-walking group only, which differentially changed when	In a free-living setting, continuous-walking exercise may offset the deleterious effects of no exercise, while interval-walking exercise may superiorly improve measures of glucose	Strengths: Extended exercise intervention; inclusion of a control group; applicable to a free-living condition for exercise and glucose control Limitations: Small sample size; limited to type

	Habitual daily activity; continuous-walking; interval-walking group	compared to the continuous-waling group.	concentrations in type 2 diabetic adults.	2diabetic adults
Francois ME et al. (2017)	Proof-of-concept, double-blind, randomized clinical trial; N=53 3 days per week for 12 weeks of high-intensity interval training (HIIT); resistance and aerobic-based exercised	There was a significant decrease in glycemic control assessed as HbA1c, as well as 24-hour mean glucose concentration, SD of the 24-hour mean glucose concentration, and MAGE.	Twelve weeks of low-volume HIIT improved glycemic control and glycemic variability.	Strengths: Standardized 12-week HIIT exercise program Limitations: Older sample limited to type 2 diabetic adults; unable to account for participant characteristics differences

Table 4.4. presents studies that provided information regarding the influence of exercise training on glycemic control and glycemic variability in type 2 diabetic adults. The table includes: 1) author information; 2) study design; 3) findings related to the alterations in glycemic control and glycemic variability; 4) conclusions derived from the findings on changes in glycemic control and glycemic variability; 5) strength and limitations of each study.

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CHAPTER 5

MANUSCRIPT 2-ASSOCATION OF SEDENTARY TIME AND PHYSICAL ACTIVITY WITH GLYCEMIC VARIABILITY AND OXIDATIVE STRESS IN OVERWEIGHT OR OBESE ADULTS[2]

[2]Sparks, JR, Sarzynski, MA, Davis, JM, Grandjean PW, & Wang, X. To be submitted to *Translational Journal of the American College of Sports Medicine*

ABSTRACT

Introduction: Overweight or obese adults have a higher risk for the development of cardiometabolic disorders. Glycemic variability acts as a more sensitive analysis of glycemic health compared to other clinical measures. Oxidative stress plays a key role in the development of cardiovascular disease. Decreased sedentary time and increased physical activity (PA) reduce the risk of cardiometabolic diseases. The purpose of this study was to examine the relationship between sedentary time and PA measures with glycemic variability and oxidative stress in overweight or obese adults.

Methods: Adults (n=28; BMI=32.3±6.3 kg/m^2) completed 7-day accelerometer and continuous glucose monitor (CGM) monitoring. Time spent sedentary and performing light- (LPA), moderate-to-vigorous-intensity (MVPA), and total PA, as well as associated energy expenditure (EE) were measured using a Sensewear Mini Armband. The average daily sedentary time, PA minutes, and PA EE per day were calculated. The standard deviation (SD) across all days for these PA variables were used to indicate the consistency of PA across days. The 24-hour glycemic variability was calculated as mean amplitude of glycemic excursion (MAGE), continuous overlapping net glycemic action of 1-hour, 2-hour, and 4-hour (CONGA-1, -2, and -4) and mean of daily differences (MODD). After a 12-hour fast, a blood sample was collected on either the first or last day of the monitoring period and concentrations of nitric oxide and myeloperoxidase were measured, and the oxidative stress ratio was calculated as nitric oxide concentration÷myeloperoxidase concentration. Pearson product correlations between measures of sedentary time and PA, glucose concentrations, glycemic variability, and oxidative stress were performed with and without adjustment for age and/or BMI.

Results: MVPA was negatively correlated with diurnal glucose concentration (r=-0.42, p=0.03), while total PA was negatively correlated with 24-hour mean glucose (r=-0.41, p=0.04). LPA EE was negatively correlated with fasting glucose concentration (r=-0.41, p=0.03) and diurnal glucose concentration (r=-0.41, p=0.03), while LPA and MVPA EE were negatively correlated with diurnal glucose concentration (r=-0.42, p=0.03; r=-0.41, p=0.04). Additionally, the SD across average daily LPA EE negatively correlated with the oxidative ratio (r=-0.39, p=0.04). Nitric oxide was positively correlated with fasting glucose concentration (r=0.47, p=0.01), while myeloperoxidase was negatively correlated with fasting glucose concentration (r=-0.41, p=0.03), MAGE (r=-0.46; p=0.02), CONGA-2 (r=-0.39, p=0.04), and CONGA-4 (r=-0.38, p=0.04). After adjustment for age, BMI, and/or time spent sedentary and performing PA, myeloperoxidase continued to be negatively correlated with fasting glucose concentration and MAGE.

Conclusion: PA of varying intensities, regardless of PA consistency, may impact glycemic health. The relationship between glycemic variability and oxidative stress may be more pertinent when evaluating cardiometabolic health, rather than the relationship between PA measures with these outcomes, in overweight or obese adults.

INTRODUCTION

The prevalence of adults in the United States classified as overweight or obese continues to increase, with ~40% designated obese in 2015-2016, and is widely considered a major public health crisis of the current generation (Flegal MF, Kruszon-Moran D, Carroll MD, Fryar CD, & Ogden CL, 2016; Hales CM, Carroll MD, Fryar CD, & Ogden CL, 2017). Overweight or obese adults are at an increased risk for the development of type 2 diabetes mellitus and cardiovascular disease (National Institutes of Health 1998; National Institutes of Health 2013; Centers for Disease Control and Prevention 2017). Further, overweight or obesity-related cardiometabolic disorders may lead to increased medical care cost to prevent or treat potential preventable disorders, such as impaired glycemic health or cardiovascular disease risk factors (Bray GA 2004; O'donovan G, Kearney EM, Nevill AM, Woolf-May K, & Bird SR, 2005).

It is suggested that overweight and obesity may not directly translate to increased risk of cardiovascular disease, rather it may be the interaction of obesity-related insulin resistance causing pathophysiological changes in the cardiovascular system that lead to cardiovascular disease (Reaven G 2005). Further, oxidative stress increases the risk for development of cardiovascular disease and has been shown to be elevated in overweight and obesity (Alberti K & Zimmet PZ, 1998; Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, & Chamari M, 2007; Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, & Arrigo T, 2015). When an imbalance between accumulation and detoxification of reactive oxygen species arises, an induction of oxidative stress occurs, resulting in vascular endothelium-related vasoconstriction (Wu JQ, Kosten TR, & Zhang XY, 2013; Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito

F, Altavilla D, & Bitto A, 2017). Hyperglycemia has been mechanistically related to increased production of oxidative stress, as increases in glucose concentrations are a known cause of enhanced free radical activation and elevated reactive oxygen species (Ceriello A 2000).

Additionally, glycemic variability has been utilized to determine glycemic control, including glucose excursions, and potentially acts as a more sensitive analysis of glycemic health compared to clinical assessments (Monnier L, Colette C, & Owens DR, 2008). Frequent or exacerbated fluctuations of glucose concentrations may contribute to deleterious complications linked to impaired glucose metabolism, and has been found to be increased in overweight or obese adults (Wang S, Lv L, Yang Y, Chen D, Liu G, Chen L, Song Y, He L, Li X, Tian H, Jia W, & Ran X, 2012; Satya Krishna SV, Kota SK, & Modi KD, 2013; Salkind SJ, Huizenga R, Fonda SJ, Walker MS, & Vigersky RA, 2014; Suh S & Kim JH, 2015). Furthermore, glycemic variability has also been found to be positively associated with oxidative stress in type 2 diabetes mellitus (Taniyama Y & Griendling KK, 2003; Monnier L, Mas E, & Ginet C, 2006; Rodrigues R, de Medeiros LA, Cunha LM, Garrote-Filho MDS, Bernardino Neto M, Jorge PT, Resende ES, & Penha-Silva N, 2018). As technology has continued to advance, free-living glycemic variability assessment has become a minimally invasive procedure utilizing continuous glucose monitoring (CGM).

Overweight or obese adults tend to spend more time being sedentary and less time performing physical activity (PA) than healthy weight adults (Tudor-Locke C, Brashear MM, Johnson WD, & Katzmarzyk PT, 2010; Ortega FB, Artero EG, Jiménez-Pavón D, & Ruiz JR, 2018). Evidence indicates that decreasing sedentary time and increasing PA

of any intensity is beneficial for cardiometabolic health, including decreases in body mass index (BMI), dyslipidemia, and fasting glucose concentrations (Chan CB, Ryan DAJ, & Tudor-Locke CT, 2004; Van Gaal FL & Maggioni AP, 2013; Wing RR, Bolin P, Brancati FL, Bray GA, Clark JM, Coday M, Crow RS, Curtis JM, Egan CM, Espeland MA, Evans M, & The Look AHEAD Research Group, 2013).

Objectively-measured sedentary time, light-intensity PA (LPA), and moderate-to-vigorous-intensity PA (MVPA) are each independently associated with 2-hour glucose concentration in overweight adults not diagnosed with type 2 diabetes mellitus (Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, & Owen N, 2007). Yet, no study has reported sedentary time and PA of varying intensities with glycemic variability. Additionally, subjectively evaluated light-intensity, high-intensity, and total leisure-time PA were associated with high antioxidant enzyme activity, which decreases oxidative stress (Covas M-I, Elosua R, Fitó M, Alcántara M, Coca L, & Marrugat J, 2002). However, whether these associations exist using objectively measured sedentary time and PA are unclear.

Therefore, the purpose of this study was to examine the associations of time spent sedentary time and performing PA and related PA energy expenditure (EE) with measures of glycemic variability and biological markers of oxidative stress in sedentary, overweight or obese adults. The overarching hypothesis was that less time spent sedentary time and more time performing PA of any intensity would be associated with better measures of glycemic variability and oxidative stress. A secondary hypothesis was that decreased glycemic variability would be associated with lower oxidative stress biological markers.

METHODS

Data were obtained from the Weight Outlooks by Restriction of Diet and Sleep (WORDS; NCT: 02413866) and Aerobic Treadmill Exercise and Metabolism (A-TEAM; NCT: 03162991) studies. The WORDS study openly recruited from January 2015 to October 2016, while the A-TEAM study openly recruited from October 2017 to December 2018. The WORDS study was designed to examine the effects of chronic moderate sleep restriction on body composition in individuals undergoing a hypocaloric dietary weight loss program (Wang X, Sparks JR, Bowyer KP, & Youngstedt SD, 2018). The A-TEAM study was designed to examine the effects of a moderate-intensity aerobic treadmill-based exercise intervention on glucose concentrations utilizing CGM technology in sedentary, overweight or obese adults. Study protocols were approved by the University of South Carolina Institutional Review Board and all participants signed an informed consent form prior to participation. There were no repeat participants between the two studies. The present study includes 28 participants that had valid CGM and PA data, as well as fasting blood samples available for analysis at baseline, prior to either study's intervention. All participant visits and testing were completed by the same trained research staff and took place in the Clinical Exercise Research Center (CERC) housed within the Norman J. Arnold School of Public Health at the University of South Carolina.

Participants

Both the WORDS and A-TEAM studies had similar inclusion and exclusion criteria with the WORDS study criteria previously described (Wang X, Sparks JR, Bowyer KP, & Youngstedt SD, 2018). Briefly, participants were not physically active

(reported <120 minutes of resistance or endurance exercise per week during the previous 3 months), overweight or obese (25≤BMI≤40 kg/m^2) males and females, age 35-55 years, weight stable (±2%) during the previous 3 months, and females were eumenorrheic or post-menopausal for ≥1 year. Exclusion criteria included any self-reported medical conditions such as diabetes, cardiovascular diseases, chronic or recurrent respiratory conditions (e.g. uncontrolled asthma or chronic obstructive pulmonary disease), active cancer, and eating or neurological disorders, medications that affect metabolism (e.g. thyroid medications, statins), psychological issues, including but not limited to untreated depression and attention deficit disorder, excessive caffeine use (>500 mg/day), smoking during the past year, pregnant or lactating females, and/or unwillingness to provide informed consent.

Measurements

Height, Body Weight, and Body Mass Index (BMI)

Height and body weight were measured at the first baseline visit using a stadiometer and an electronic scale that was calibrated annually (CC Vaughan & Sons, Incorporated, Columbia, SC). BMI was calculated using the following calculation: BMI (kg/m^2) = Body Weight (kg) ÷ [Height (m)]^2.

Sedentary Time and Physical Activity Measures

At the first baseline visit, participants reported to the CERC and were trained on use of the SenseWear Mini Armband device (BodyMedia®, Pittsburgh, PA), which was initially placed by trained research staff on the posterior aspect of the left arm at the mid-point between the olecranon and acromion processes. The SenseWear device provides objectively measured PA and has demonstrated to be reliable and valid when measuring

energy expenditure compared to the doubly labeled water method (Johannsen DL, Calabro MA, Stewart J, Franke W, Rood JC, & Welk GJ, 2010) and more accurate than the standard World Health Organization Global Physical Activity Questionnaire for estimating PA (Laermans M, Dons E, Avila-Palencia I, Carrasco-Turigas G, Orjuela JP, Anaya E, Brand C, Cole-Hunter T, de Nazelle A, Götschi T, Kahlmeier S, Nieuwenhuijsen M, Standaert A, Boever PD, & Panis LI, 2017). Participants wore the device for 7 consecutive days, before either study intervention, and were instructed to maintain their normal daily routine during monitoring. Participants recorded when they removed the device for any activities that required water-based activities, such as showering/bathing or swimming over the 7-day monitoring period. On the final day of the 7-day monitoring period, participants reported back to the CERC for their second baseline visit and returned the SenseWear device. Data were considered valid for analysis if participants wore the monitor for at least 5 days including at least one weekend day, with a minimum wear time of 20 hours each day.

Sedentary time and PA measures were assessed utilizing manufacture provided software (BodyMedia® Sensewear Version 7.0). Sedentary time was established as activity <1.5 metabolic equivalents (METs) (excluding sleep time), and time spent performing LPA as 1.5 to <3.0 METs, MVPA as ≥3.0 METs, and total PA as ≥1.5 METs. In addition to sedentary time and time spent performing PA of varying intensities, EE for LPA (≥1.5 METs) and MVPA (≥3.0 METs) were obtained. Sedentary time and time spent performing PA of varying intensity, LPA EE, and MVPA EE were obtained for each valid day of wear time and the average of those days were calculated. Additionally, the standard deviation (SD) of sedentary time and all PA measures across all valid days

were calculated as a measure of day-to-day variability. Estimated PA time and EE during non-wear times was excluded from analysis.

CGM-Assessed Glucose Concentrations and Glycemic Variability

A CGM device (Dexcom G4 Platinum Professional, San Diego, CA, USA) was used to assess interstitial glucose concentrations throughout the same 7 consecutive days as the SenseWear Mini Armband device monitoring. At the first baseline visit participants reported to the CERC for placement and instruction of use for the CGM device by trained research staff. Participants had a catheter inserted under the skin on the preferred side of the abdomen with an attached sensor and transmitter, approximately 2 cm to the side of the umbilicus. They were instructed to carry a recording device which received and stored interstitial glucose concentration readings every 5 minutes over the 7 days. This specific CGM device model requires participants to manually perform a capillary blood measurement by fingerstick using a provided glucometer twice a day during days of wear per manufacture instructions. Participants were instructed on how to perform a capillary blood measurement and enter the glucometer readings into the CGM device. The Dexcom G4 Platinum Professional CGM device has been validated and proven accurate against directly evaluated blood glucose concentrations (Facchinetti A, Favero SD, Sparacubi G, & Cobelli C, 2015). The CGM device was blinded so that participants could not observe the live readings to deter any alterations in diet, PA, or general lifestyle, and participants were requested to maintain their normal daily routine during the 7-day monitoring period. On the final day of the 7-day monitoring period, participants reported back to the CERC for their second baseline visit and the CGM device was removed. Data was considered valid for analysis if participants wore the

device for at least 5 days including at least one weekend day, with a minimum available glucose measure over 20 hours each day.

Software provided by the manufacturer (Dexcom Studio 12.0.4.6) was used to download and export CGM data. 24-hour mean, diurnal, and nocturnal glucose concentrations were calculated for each valid day and expressed as the average of those valid days. 24-hour mean glucose concentration was assessed from midnight to midnight for each valid day. Diurnal and nocturnal glucose concentrations were assessed each valid day during each participant's self-reported time-in-bed and time-out of-bed. The continuous overlapping net glycemic action over 1, 2, and 4 hours (CONGA-1, CONGA-2, and CONGA-4) was calculated manually in Excel, while the mean amplitude of glycemic excursion (MAGE) and mean of daily differences (MODD) measures of glycemic variability was calculated using EasyGV Version 9.0.R2 (University of Oxford, Oxford, England, UK), which is an Excel-enabled workbook that utilizes macros. MAGE was calculated for each participant by taking the arithmetic mean of increased or decreased glucose concentrations (nadirs and peaks or vice-versa) when both ascending and descending concentration exceeds one SD from the 24-hour mean glucose concentration for the same 24-hour monitoring period (Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, & Taylor WF, 1970; Service FJ & Nelson RJ, 1980; Service FJ, O'Brien PC, & Rizza RA, 1987). CONGA-1, CONGA-2, and CONGA-4 were calculated as the SD of the differences between each observation and the previous 1-hour, 2-hour, and 4-hour observations (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005; Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, & ADAG Study Group, 2008; Kuenen JC, Borg R, Zheng H, Schoenfeld D,

Heine RJ, & Nathan DM, 2011). CONGA-1, CONGA-2, and CONGA-4 were chosen for this study because the 1-hour, 2-hour, and 4-hour time periods approximate the time intervals between different activities (CONGA-1), time between snacks (CONGA-2), and time between meals (CONGA-4), respectively (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005). MODD accounts for the mean of absolute differences between glucose concentrations obtained at exactly the same time of day on consecutive days and has been highly correlated with the SD for between day variability (Rodbard D, Matsubara B, Nakamura K, Bailey T, Jovanovic L, Zisser H, Kaplan R, & Garg SR, 2008). MAGE and CONGA-1, CONGA-2, and CONGA-4 were utilized as measurements of within-day glycemic variability for each valid day of wear time and averages of those days calculated, while MODD was utilized as a measurement of between-day glycemic variability for all valid days combined.

Venous Blood Sample Collection

For the WORDS study, participants reported to the CERC and had a fasting blood sample collected following a minimum of a 12-hour fast (not including water) at the first baseline visit (day 1). Fasting blood samples were collected into a BD Vacutainer serum collection tube, allowed to clot for approximately 30 minutes, and then centrifuged at 3000 rpm at 4ºC for 20 minutes. For the A-TEAM study, participants reported to the CERC following a minimum of a 12-hour fast (not including water) at the second baseline visit (day 7) and had a blood sample collected into a BD Vacutainer EDTA plasma collection tube, which was immediately centrifuged at 3000 rpm at 4ºC for 20 minutes. Serum separated after centrifugation was aliquoted and stored at -80ºC until all participants' samples were ready for analysis. Prior to analysis, serum and plasma

samples were thawed and re-centrifuged at 3000 rpm and 4ºC for 20 minutes to ensure separation of any particulate.

Fasting Glucose Measurement

A YSI 2300 STAT Plus (YSI Life Sciences, Yellow Spring, OH), which was calibrated according to manufacture instruction prior to analysis, was used to analyze serum glucose. The time of blood sample collection was recorded and matched with the CGM to establish the fasting glucose time point. If the fasting blood sample time point fell between CGM glucose concentration readings, as the CGM assesses glucose concentrations every 5 minutes, the average between the previous and following CGM concentration readings was calculated. The CGM-assessed glucose concentrations have been validated with venous blood glucose concentrations, which maintained an accuracy rate between 95-99% during periods of euglycemia (Kovatchev B, Anderson S, Heinemann L, & Clarke W, 2008).

Biological Markers of Oxidative Stress

Fasting venous blood samples were used to measure biological markers of oxidative stress. Two biological markers of oxidative stress, nitric oxide, which acts as a potent vasodilator (Palmer RMJ, Ashton DS, & Moncada S, 1988), and myeloperoxidase, a potent vasoconstrictor (Klebanoff SJ 2005), were measured using two separate enzyme-linked immunoabsorbant assays (ELISA). The nitric oxide ELISA kit (ThermoFisher Scientific, Waltham, MA) quantitatively determines the concentrations of nitrate and nitrite, with ≥90% sample recovery rate, in serum and plasma samples. The myeloperoxidase ELISA kit (Eagle Biosciences, Inc., Nashua, NH) quantifies myeloperoxidase utilizing a two-site “sandwich” technique that binds to different

epitopes of myeloperoxidase. However, myeloperoxidase concentrations have been observed to be lower in plasma compared to serum based on previous tests performed by the manufacturer. The oxidative stress ratio was calculated by the concentration of nitric oxide divided by the concentration of myeloperoxidase to examine balance between vasodilation (nitric oxide) and vasoconstriction (myeloperoxidase). All assays were performed and analyzed on the same day by the same trained research staff with an intra-assay variability of <10%.

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 (Cary, NC). Participant characteristics were calculated and reported as mean and SD as a combined sample and for each study. Descriptive statistics were calculated for sedentary time and PA of varying intensity, measures of glycemic variability, and biological markers of oxidative stress. Participant characteristics for each study were compared utilizing independent sample t-tests, or chi-square test when necessary, to determine whether any variables were different between the two studies. Pearson product correlations were performed to examine the associations between sedentary time and PA measures, fasting and CGM glucose concentrations, glycemic variability, and biological markers of oxidative stress. Furthermore, the relationship between glucose concentrations, glycemic variability measures, and biological markers of oxidative stress with the day-to-day variability in sedentary time and PA measures, expressed as SD, was examined. Effect size correlations were based upon standards set at r=0.1 (-0.1), 0.3 (-0.3), or 0.5 (-0.5), representing a small, medium, or large effect size correlation, respectively (Cohen J, 1988). Partial Pearson product correlations adjusting for age and/or BMI were performed

to determine their influence on the relationship between the outcome variables of interest. Additionally, as time spent sedentary or performing varying intensities of PA were primary outcomes of interest for this study, adjustments for these measures were performed when evaluating the relationship between measures of glycemic variability and biological markers of oxidative stress. A p value of <0.05 was considered statistically significant.

RESULTS

Participant Characteristics

Participant characteristics are shown in Table 5.1 for all participants and separately for the WORDS and A-TEAM studies. Overall, participants were approximately 70% female and 57% African American with 54% considered obese (BMI $\geq$30 kg/m^2). Between the studies, participants only differed in the oxidative stress ratio ($p=0.03$), but did not differ for any participant characteristic, anthropometric, PA, glucose concentration, glycemic variability measurements, or concentration of oxidative stress biological markers ($p\geq0.05$; Table 5.1.).

Association between Sedentary Time and Physical Activity Measures with Glucose Concentrations, Measure of Glycemic variability, and Biological Markers of Oxidative Stress

The correlation between sedentary time and PA measures with glucose concentrations, measures of glycemic variability, and biological markers of oxidative stress are shown in Table 5.2. We observed negative correlations between LPA EE and fasting and diurnal glucose concentrations, MVPA minutes and EE with diurnal glucose concentration, and total PA minutes with 24-hour mean glucose concentration. In addition, several other negative correlations between PA variables and glucose concentrations and glycemic variability measures were of medium effect size (absolute r

value>0.30), although statistically not significant (0.05<p<0.10), such as LPA and MVPA minutes and EE with 24-hour mean glucose (0.05<p≤0.07 for all).

Following adjustment for age alone, negative correlations remained between LPA EE and MVPA minutes and EE with diurnal glucose concentration, while negative correlations between MVPA minutes and 24-hour mean glucose concentrations were found (p<0.05 for all). However, after adjusting for BMI alone, all prior significant correlations were no longer significant (p≥0.05 for all). Though, after adjustment for age and BMI combined, LPA EE was found to be negatively correlated with diurnal glucose concentrations (r=-0.43, p=0.04).

There were no significant correlations between sedentary time or any PA measure with any glycemic variability measure (Table 5.2.). However, there were medium effect size negative correlations between minutes of LPA and total PA with MAGE and CONGA-4, while LPA EE also expressed a medium effect size negative correlation with MAGE and CONGA-2 (0.31≤absolute r≤0.33, 0.10≤p≤0.14 for all). Yet, there were no significant correlations between sedentary time and PA measures with concentrations of nitric oxide or myeloperoxidase, which persisted after adjustment for age and/or BMI (p≥0.06 for all).

We further examined the day-today variability in sedentary time and PA measures expressed as the SD across valid SenseWear monitoring days with glucose concentrations, glycemic variability, and oxidative stress, which is shown in table 5.3. Interestingly, there were significant negative correlations between the SD of MVPA and total PA minutes, as well as LPA and MVPA EE, with 24-hour mean, diurnal, and nocturnal glucose concentrations (0.40≤absolute r≤0.53, p≤0.04 for all). Additionally,

medium effect size correlations between the SD of MVPA minutes, LPA and MVPA EE with MODD were observed, which persisted following adjustment for age and/or BMI (absolute $r \geq 0.30$ for all). However, there was a significant negative correlation between SD of LPA EE with the oxidative stress ratio ($r=-0.39$, $p=0.04$).

Association between Glucose concentrations and Measures of Glycemic Variability with Biological Markers of Oxidative Stress

Correlations between glucose concentrations and measures of glycemic variability with biological markers of oxidative stress are shown in Table 5.4. When examining the relationship between glucose concentrations with biological markers of oxidative stress, nitric oxide concentration and the oxidative stress ratio were found to be positively correlated with fasting glucose concentration, which persisted after adjustment for age or BMI ($p \leq 0.04$ for both), but no longer significant following adjustment for age and BMI ($p=0.07$). Myeloperoxidase concentration was found to be negatively correlated with fasting glucose concentration ($r=-0.41$, $p=0.03$), which persisted after adjustment for age and/or BMI ($p \leq 0.03$ for all). Further, the oxidative stress ratio positively correlated with fasting glucose concentration ($r=0.60$, $p \leq 0.001$), which persisted after adjustment for age and/or BMI ($p \leq 0.004$ for all).

Furthermore, we examined the relationship between measures of glycemic variability and biological markers of oxidative stress and found that myeloperoxidase concentration was negatively correlated with MAGE, CONGA-2 and CONGA-4, which remained following adjustment for age and/or BMI ($p \leq 0.02$ for all). However, neither nitric oxide or myeloperoxidase concentrations significantly correlated with CONGA-1 or MODD measure of glycemic variability. Yet, after adjustment for age and/or BMI in

the model, the oxidative stress ratio was positively correlated with MAGE, CONGA-1, and CONGA-2 measures of glycemic variability ($p<0.05$ for all).

Lastly, to examine the role of time spent sedentary and performing PA on the relationship between glucose concentrations and glycemic variability with biological markers of oxidative stress, adjustments for sedentary time and all intensities of PA were performed. Following this adjustment, nitric oxide and the oxidative stress ratio remained positively correlated with fasting glucose concentration ($p=0.04$ and 0.001, respectively), while myeloperoxidase remained negatively correlated with fasting glucose concentration ($p=0.02$). Moreover, a positive correlation between the oxidative stress ratio and MAGE presented itself ($p=0.03$), while myeloperoxidase remained negatively correlated with MAGE ($p=0.01$), CONGA-2 ($p=0.04$), and CONGA-4 ($p=0.04$).

DISCUSSION

To our knowledge, this is the first study to examine the relationship between objectively measured sedentary time and PA with CGM-assessed glucose concentrations and glycemic variability, and biological markers of oxidative stress in sedentary, overweight or obese adults. Notably, MVPA and total PA minutes were found to be negatively correlated with 24-hour mean and diurnal glucose concentrations, while LPA and MVPA EE were found to be negatively correlated with fasting and diurnal glucose concentrations, respectively. However, no significant correlations were found between sedentary time or PA measures with measures of glycemic variability. Yet, day-to-day variability in PA minutes and EE of varying intensities were significantly positively correlated with multiple glucose concentrations, while LPA EE negatively correlated

with the oxidative stress ratio expressed as nitric oxide concentration÷myeloperoxidase concentration.

Relationship between Sedentary Time and Physical Activity with Glucose Concentrations, Glycemic Variability, and Oxidative Stress

Impaired fasting glucose concentration and glycemic control have been observed during periods of decreased PA (Lipman RL, Raskin P, Love T, Triebwasser J, Lecocq FR, & Schnure JJ, 1972). Most previous studies examining the impact of sedentary time and PA measures with glycemic health have found beneficial effects of increasing PA of any intensity and decreasing sedentary time on varying glucose concentrations (Sardinha LB, Magalhães JP, Santos DA, & Júdice PB, 2017; Swindell N, Mackintosh K, McNarry M, Stephens JW, Sluik D, Fogelholm M, Drummen M, MacDonald I, Martinez JA, Handjieva-Darlenska T, Poppitt SD, Brand-Miller J, Larsen TM, Raben A, & Stratton G, 2018). However, few studies have examined this relationship utilizing objective, accelerometry measured sedentary time and PA. One study examined the cross-sectional associations between objectively measured sedentary and PA time with cardiometabolic disease risk biomarkers in overweight or obese adults with type 2 diabetes and found that LPA minutes per day was favorably associated with fasting glucose concentration (Healy GN, Winkler EAH, Brakenridge CL, Reeves MM, & Eakin EG, 2015). Additionally, a study conducted by Hamasaki et al., examined the association between objectively assessed daily PA with metabolic risk factors in adults with prediabetes and untreated early type 2 diabetes mellitus and found that PA level was negatively associated with fasting glucose concentration (Hamasaki H, Noda M, Moriyama S, Yoshikawa R, Katsuyama H, Sako A, Mishima S, Kakei M, Ezaki O, & Yanai H, 2015). These studies support our findings that varying intensities of PA were favorably associated with fasting

glucose concentration; however, our study further expanded the evidence to support this relationship exists with 24-hour mean, diurnal, and nocturnal glucose concentrations.

Additionally, there exists limited evidence regarding the relationship between objectively measured sedentary time and PA with glycemic variability. One cross-sectional study performed by Gude et al., found that glycemic variability indices, which included MAGE, CONGA-1, and MODD, were not associated with subjective assessment of time spent sedentary or performing PA, utilizing the International Physical Activity Questionnaire, in adults without type 2 diabetes mellitus, regardless of categorized PA status, which included inactive, minimally active, and high active categories (Gude F, Díaz-Vidal P, Rúa-Pérez C, Alonso-Sampedro M, Fernández-Merino C, Rey-García J, Cadarso-Suárez C, Pazos-Couselo M, García-López JM, Gonzalez-Quintela A, 2017). Another study performed in type 1 diabetic adults found that objectively assessed total PA time did not significantly correlate with glycemic variability assessed as the standard deviation of the 24-hour mean glucose concentration (Martyn-Nemeth P, Quinn L, Penckofer S, Park C, Hofer V, & Burke L, 2017). Therefore, these studies are in line with our findings that increases in PA and their related PA EE are not necessarily related to improvement in glycemic variability. However, though in line with our findings, the inclusion of older adults, utilization of adults with diagnosed type 1 and 2 diabetes, or the use of subjective assessment of time spent sedentary and performing PA, do not allow for direct translation of their findings to ours.

Previous literature has expressed that decreases in sedentary time and increases in leisure-time PA of varying intensities relate to improvements in antioxidants known to decrease oxidative stress (Thosar SS, Johnson BD, Johnston JD, & Wallace JP, 2012).

Additionally, increases in sedentary behavior have been linked to decreases in endothelium-derived nitric oxide vasodilation (Demiot C, Dignat-George F, Fortrat JO, Sabatier F, Gharib C, Larina I, Gauquelin-Koch G, Hughson R, & Custaud MA, 2007). In our study, no measure of sedentary time or PA of any intensity were associated with nitric oxide or myeloperoxidase concentration, or the oxidative stress ratio. A previous study examined the relationship between subjective leisure-time PA EE (Met-minutes/day) with antioxidants related to lower oxidative stress in adult females and found that low-intensity, high-intensity, and total-intensity PA EE were positively associated with antioxidant concentration (Covas M-I, Elosua R, Fitó M, Alcántara M, Coca L, & Marrugat J, 2002). This illustrates that PA of varying intensities may be related to decreases in oxidative stress; however, their study utilized subjective evaluation of PA which has been shown to be unreliable for reproducibility (Sallis JF & Saelens BE, 2000), rather than objective accelerometer-based measurements, which provide a more valid examination of this relationship.

Unexpectedly, increased day-to-day variability across daily average PA and EE of varying intensities were significantly related to decreases in glucose concentrations, as well as expressed medium effect size, though insignificant, relationships with MODD. These findings suggest that increases in day-to-day variability in PA relate to decreased glucose concentrations and inter-day glycemic variability. However, increases in day-to-day PA variability related to a decrease in the oxidative stress ratio, which suggests that decreased variability in PA relates to lower oxidative stress.

Most studies have primarily examined day-to-day variability to assess habitual sedentary time and PA, as multiple days are required, but have not focused on health

outcomes related to these variability measures (Baranowski T & de Moor C, 2000). Additionally, previous research suggests it is difficult to account for the day-to-day variability in sedentary time and PA in adults, but has observed that stability or variability in daily PA and their subsequent associations with aspects of physical health, such as chronic disease and mortality, is equivocal (Maher JP, Huh J, Intille S, Hedeker D, & Dunton GF, 2018). Data from the Harvard Alumni Health Study found that men, without major risk factors, who sporadically engaged in PA (1-2 days per week), but met recommended levels of PA (≥1000 kcal per week), had a lower relative risk of mortality compared to men who were considered inactive, but similar to those considered insufficiently active (500-999 kcal per week) or regularly active (≥1000 kcal per week; >2 days per week) (Lee IM, Sesso HD, Oguma Y, & Paffenbarger Jr RS, 2004). Moreover, a longitudinal study which examined insufficiently active, sporadically active, and regularly active participants found similar reductions in risk for all-cause and cardiovascular disease mortality compared to inactive participants (O'Donovan G, Lee IM, Hamer M, & Stamatakis E, 2017). Therefore, the stability or variability in PA may not directly relate to clinical implications in health outcomes associated with glycemic or cardiovascular health if adherence to prevailing PA guidelines are fulfilled. As the participants in our study were achieving on average ~65.5 minutes of MVPA per day during the 7 days of SenseWear wear time, it may be generally accepted they were meeting current PA guidelines (Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, George SM, & Olson RD, 2018) regardless of the extent of their day-to-day PA variability. Even though there was notable day-to-day variability in sedentary time and PA, higher PA variability may indicate more PA being performed on certain

days as opposed to increased sedentary time on others. This aids in supporting our findings that general participation in PA may potentially be more important for glycemic health, rather than PA stability.

Relationship between Glucose Concentrations and Glycemic Variability with Oxidative Stress

The relationship between glucose concentrations and glycemic variability with oxidative stress has been evaluated primarily in type 1 and type 2 diabetics (Saisho Y 2014). Additionally, it has been shown that there exists long-term vascular complications in diabetic patients during periods of chronic hyperglycemia, as well as those with greater postprandial glucose fluctuations (Giugliana D, Ceriello A, & Paolisso G, 1996; Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol J-P, & Colette C, 2006). However, no studies have examined the relationship between glucose concentrations, glycemic variability, and oxidative stress in sedentary, overweight or obese, but normoglycemic adults.

Surprisingly, our study found that nitric oxide concentration, a potent vasodilator and measure of vascular health, was positively associated, rather than negatively associated, with fasting glucose concentration. Current evidence suggests contraindicatory findings when examining the relationship between fasting glucose and nitric oxide concentrations. It has been hypothesized that chronic hyperglycemia acts to uncouple receptor-mediated signal transduction and decrease availability of nitric oxide synthase substrates and cofactors essential for synthesis of nitric oxide (Honing MLH, Morrison PJ, Banga JD, Stroes ESG, & Rabelink TJ, 1998). Li et al., found that experimentally increased activation of the nitric oxide pathway partially contributed to skeletal muscle glucose uptake (Li J, Hu X, Selvakumar P, Russell III RR, Cushman SW,

Holman GD, & Young LH, 2004). Furthermore, Hoshiyama et al., found that experimentally high-level glucose exposure reduced nitrite levels and subsequently decreased nitric oxide production, but upregulated endothelial cell nitric oxide synthase protein expression (Hoshiyama M, Li B, Yao J, Harada T, Morioka T, & Oite T, 2003). These studies suggest that under hyperglycemic conditions, decreased nitric oxide production may be due to reduced nitric oxide predecessors or nitric oxide synthase agonists, and increased activation of nitric oxide scavengers acting to inactivate nitric oxide synthase activity. However, Cosentino et al., provided evidence that experimentally increased glucose exposure increased nitric oxide synthase and subsequent nitric oxide release (Cosentino F, Hishikawa K, Katusic ZS, & Lüscher TF, 1997). More recently, Adela et al., observed a positive relationship between fasting blood glucose and nitric oxide concentration in patients diagnosed with type 2 diabetes mellitus, while also providing evidence that experimentally high glucose exposure increases nitric oxide production (Adela R, Nethi SK, Bagul PK, Baui AK, Mattapally S, Kuncha M, Patra CR, Reddy PNC, & Banerjee SK, 2015). These findings suggest that the mechanisms by which high glucose concentration simultaneously increases nitric oxide synthase expression and production may be due to an increase in glucose-induced endothelial cell nitric oxide gene expression and activation related to diminished vascular function in those with impaired glycemic health. Based on previous findings, along with the findings in our study, it may be hypothesized that, even in the absence of glycemic dysfunction, participants with higher fasting glucose concentration may have higher activation of nitric oxide synthase and in turn greater nitric oxide production compared to those with lower fasting glucose concentration.

Unexpectedly, myeloperoxidase concentration, a potent vasoconstrictor and measure of declined vascular health, was negatively associated with not only fasting glucose concentration, but MAGE, CONGA-2, and CONGA-4 measures of glycemic variability. The relationship between glycemic health and myeloperoxidase has been previously investigated but arrived at contradicting conclusions. Zhang et al., found that fasting glucose and myeloperoxidase concentrations were positively associated, with increases in glucose concentration directly relating to increases in myeloperoxidase concentration in non-diabetic adults with acute coronary syndrome (Zhang X, Dong L, Wang Q, & Xie X, 2015). However, Uchimura et al., found that fasting glucose concentration was higher and myeloperoxidase concentration was lower in non-insulin dependent type 2 diabetes mellitus compared to non-diabetic controls, but found no relationship between fasting glucose and myeloperoxidase concentrations (Uchimura K, Nagasaka A, Hayashi R, Makino M, Nagata M, Kakizawa H, Kobayashi T, Fujiwara K, Kato T, Iwase K, Shinohara R, Kato K, & Itoh M, 1999). Furthermore, a previous study in adult patients with type 2 diabetes mellitus who had either poor or optimal glycemic control found that those with poor glycemic control had decreased myeloperoxidase activity compared to those with optimal glycemic control (Unubol M, Yavasoglu I, Kacar F, Guney E, Omurlu IK, Ture M, Kadikoylu G, & Bolaman Z, 2015). The authors concluded that decreases in myeloperoxidase activity found in adults with non-insulin dependent type 2 diabetes mellitus may be caused by diabetic complications due to the altered state of free radical concentrations. Additionally, increased protein glycation in type 2 diabetes patients with poor glycemic control may contribute to hyperglycemic-associated negative modulating enzymatic activity and loss of physiological function,

which may subsequently lead to decreased myeloperoxidase, as previously described (Brownlee M 2001; de Souza Ferreira C, Araújo TH, Ángelo ML, Pennacchi PC, Okada SS, de Araújo Paula FB, Migliorini S, & Rodrigues MR, 2012). Thus, our results are consistent with some of the literature; however, our participants were normoglycemic and most previous literature have suggested these findings in adults with cardiovascular complications or diagnosed with type 2 diabetes mellitus.

Strengths and Limitations

The primary strengths of this study include the use of the SenseWear Mini Armband accelerometer to objectively measure sedentary time and PA, as well as CGM technology to assess glycemic variability, which allows for the observation of a "free-living" condition as opposed to standard clinical measures. The primary limitation is that the participants were from a convenience sample, which makes the results only generalizable to sedentary, overweight or obese individuals without overt diabetes that are between the ages of 35 and 55 years. Another limitation was that diet was not fully taken into account, as only self-report dietary intake was provided. However, we recommended that each participant attempt to maintain their daily dietary routines during the 7-day monitoring process. Alternatively, this was also considered a strength of the study, as not controlling for diet provides insight into each participant's "free-living" glucose concentrations and glycemic variability. Other limitations include the small sample size, which does not allow for analysis between sexes and races that may be pertinent to address the relationships between outcomes of interest related to sedentary time and PA with glycemic variability and oxidative stress.

Conclusion

Our hypotheses were supported as there were significant relationships between MVPA and total PA minutes with diurnal and 24-hour mean glucose concentrations, LPA EE with fasting and diurnal glucose concentrations, and MVPA EE with diurnal glucose concentrations. Additionally, medium effect size correlations were found between time spent performing LPA and total PA with MAGE and CONGA-4 and LPA EE with MAGE and CONGA-2. However, no significant relationships were found between sedentary time or PA measures with nitric oxide or oxidative stress. Further, the SD of daily MVPA and total PA minutes, and LPA and MVPA EE were negatively associated with 24-hour, diurnal, and nocturnal glucose concentrations, while the SD of LPA EE was found to be negatively correlated with the oxidative stress ratio. Additionally, nitric oxide, myeloperoxidase, and the oxidative stress ratio were found to be associated with fasting glucose concentration, while myeloperoxidase was found to be associated with MAGE, CONGA-2, and CONGA-4. After adjustment for age and/or BMI, as well as time spent sedentary or performing PA, many of the relationships remained significant, which leads us to support our claims that sedentary time and participation in PA, regardless of PA consistency, play a vital role in glycemic health, while glycemic variability is related to oxidative stress.

Table 5.1. Participant characteristics combined and by study involvement

	Combined (n=28)	WORDS (n=15)	A-TEAM (n=13)
Participant characteristics			
Age (years)	46.0±6.1	45.4±6.5	46.7±5.8
Sex (M/F)	8/20	3/12	5/8
Race (C/AA/AAA)	11/16/1	8/7/0	3/9/1
Height (m)	1.7±0.1	1.7±0.1	1.7±0.1
Body Weight (kg)	92.6±21.4	93.3±12.0	91.8±29.3
Body Mass Index (kg/m^2)	32.3±6.3	32.6±3.9	31.9±8.4
Average daily sedentary time and physical activity measures			
Wear time (hours per day)	23.4±0.5	23.4±0.5	23.4±0.6
Sedentary Time (minutes per day)	717.2±124.0	717.3±61.5	717.2±170.9
LPA (minutes per day)	247.0±94.7	249.5±81.9	244.3±110.2
MVPA (minutes per day)	65.5±42.2	74.6±47.0	55.7±35.6
Total PA (minutes per day)	312.5±121.8	321.1±109.3	300.0±137.4
LPA EE (kilojoules per day)	4405.9±1728.6	4958.5±2015.8	3810.7±1154.2
MVPA EE (kilojoules per day)	1605.9±1230.0	1962.4±1505.4	1221.9±717.5
Day-to-day variability across average daily sedentary time and physical activity measures			
Sedentary Time SD (minutes per day)	113.8±65.8	101.0±36.2	127.6±86.9
LPA SD (minutes per day)	61.4±29.1	59.7±29.0	63.2±30.3
MVPA SD (minutes per day)	32.5±24.3	38.9±30.5	25.6±13.2
Total PA SD (minutes per day)	74.5±36.6	73.2±35.4	75.8±39.2
LPA EE SD (kilojoules per day)	1248.1±751.6	1336.8±908.1	1152.6±558.2

MVPA EE SD (kilojoules per day)	854.0±750.9	1072.2±958.8	619.1±332.4
Glucose concentrations and measures of glycemic variability			
CGM observations per day (n; % of max)	276.2±7.8; 96%	277.2±7.3; 96%	275.2±8.5; 95%
Fasting (mg/dL)	89.1±18.3	84.8±19.3	94.1±16.4
24-hour mean (mg/dL)	101.2±16.7	99.4±12.1	103.0±21.0
Diurnal (mg/dL)	101.8±16.0	99.0±11.2	104.7±20.0
Nocturnal (mg/dL)	103.0±16.0	103.1±10.1	102.9±21.1
MAGE (mg/dL)	43.0±12.1	40.3±12.8	45.9±11.1
CONGA-1 (mg/dL)	19.3±5.0	18.6±5.5	20.0±4.4
CONGA-2 (mg/dL)	23.3±6.3	22.5±6.9	24.1±5.6
CONGA-4 (mg/dL)	25.5±6.6	24.4±7.0	26.1±6.3
MODD (mg/dL)	19.9±4.5	19.5±4.1	20.3±4.9
Oxidative stress biological markers			
Nitric oxide (µmol/L)	75.6±40.9	62.3±47.8	91.0±25.0
Myeloperoxidase (ng/mL)	25.2±8.2	26.8±8.7	23.2±7.6
Oxidative stress ratio (µmol/L:ng/mL)	3.3:1	2.6:1	4.2:1*

Data presented as Mean±SD
M=male, F=female, C=Caucasian, AA=African American, AAA=Asian/Asian American, PA=physical activity, LPA=light-intensity physical activity, MVPA=moderate-to-vigorous-intensity physical activity, LPA EE=light-intensity physical activity energy expenditure, MVPA EE=moderate-to-vigorous-intensity physical activity energy expenditure, number of observations per day (n; % of max)=(number of CGM observations per day/maximum observations per day)×100, CONGA-1, 2, and 4=continuous overall net glycemic action of 1-hour, 2-hour, and 4-hour, MAGE=mean amplitude of glycemic excursion, MODD=mean of daily differences, oxidative stress ratio=nitric oxide concentration (µmol/L)÷myeloperoxidase concentration (ng/mL)
*$p<0.05$ significant difference between studies

Table 5.2. Pearson product correlations between average daily sedentary time and physical activity measures with glucose concentrations, measures of glycemic variability, and biological markers of oxidative stress

	Time spent sedentary time performing and physical activity (minutes per day)				*Energy expenditure (kilojoules per day)*	
	Sedentary	LPA	MVPA	Total PA	LPA EE	MVPA EE
Glucose concentrations (mg/dL)						
Fasting	0.25 (0.20)	-0.31 (0.12)	-0.36 (0.06)	-0.36 (0.06)	**-0.41 (0.03)**	-0.35 (0.07)
24-hour mean	0.28 (0.16)	-0.37 (0.07)	-0.39* (0.05)	**-0.41 (0.04)**	-0.37 (0.06)	-0.36 (0.07)
Diurnal	0.25 (0.22)	-0.30 (0.13)	**-0.42*** **(0.03)**	-0.36 (0.07)	**-0.42*‡ (0.03)**	**-0.41* (0.04)**
Nocturnal	0.26 (0.21)	-0.34 (0.09)	-0.35 (0.08)	-0.37 (0.06)	-0.37 (0.06)	-0.32 (0.11)
Measures of glycemic variability (mg/dL)						
MAGE	0.12 (0.56)	-0.33 (0.10)	-0.18 -(0.39)	-0.31 (0.12)	-0.30 (0.14)	-0.16 (0.42)
CONGA-1	0.03 (0.87)	-0.23 (0.26)	-0.10 (0.62)	-0.21 (0.30)	-0.26 (0.19)	-0.12 (0.54)
CONGA-2	0.12 (0.56)	-0.29 (0.15)	-0.17 (0.40)	-0.28 (0.16)	-0.30 (0.14)	-0.17 (0.40)
CONGA-4	0.19 (0.34)	-0.33 (0.10)	-0.19 (0.35)	-0.32 (0.12)	-0.27 (0.19)	-0.15 (0.48)
MODD	0.07 (0.73)	-0.11 (0.59)	-0.19 (0.36)	-0.15 (0.48)	-0.13 (0.53)	-0.16 (0.43)
Oxidative stress biological markers						
Nitric oxide (μmol/L)	0.18 (0.37)	-0.11 (0.60)	-0.17 (0.39)	-0.14 (0.48)	-0.14 (0.48)	-0.10 (0.61)

Myeloperoxidase (ng/mL)	0.00 (0.99)	-0.00 (0.98)	-0.05 (0.78)	-0.02 (0.91)	0.10 (0.62)	0.07 (0.71)
Oxidative stress ratio (µmol/L:ng/mL)	0.19 (0.35)	-0.13 (0.52)	-0.12 (0.56)	-0.14 (0.48)	-0.19 (0.34)	-0.10 (0.62)

Data represented as correlation coefficient (p value) with bolded entries corresponding to significant values ($p<0.05$)
PA=physical activity, LPA=light-intensity physical activity, MVPA=moderate-to-vigorous-intensity physical activity, LPA EE=light-intensity physical activity energy expenditure, MVPA EE=moderate-to-vigorous-intensity physical activity energy expenditure, METs=metabolic equivalents
*$p<0.05$ following adjustment for age; ‡$p<0.05$ following adjustment for age and BMI

Table 5.3. Pearson product correlations between day-to-day variability, expressed as SD, across daily average sedentary time and physical activity measures with glucose concentrations, measures of glycemic variability, and biological markers of oxidative stress

	Time spent sedentary time performing and physical activity (minutes per day)				*Energy expenditure (kilojoules per day)*	
	Sedentary	LPA	MVPA	Total PA	LPA EE	MVPA EE
Glucose concentrations (mg/dL)						
Fasting	0.26 (0.20)	-0.19 (0.35)	-0.34 (0.08)	-0.22 (0.27)	-0.37 (0.06)	-0.38 (0.05)
24-hour mean	0.01 (0.95)	-0.35 (0.08)	**-0.45* (0.02)**	**-0.40 (0.04)**	**-0.49*†‡ (0.01)**	**-0.43*† (0.03)**
Diurnal	-0.09 (0.64)	-0.35 (0.08)	**-0.53*†‡ (0.01)**	**-0.43 (0.03)**	**-0.53*†‡ (0.01)**	**-0.49*†‡ (0.01)**
Nocturnal	-0.06 (0.76)	-0.37 (0.07)	**-0.48*†‡ (0.01)**	**-0.41 (0.03)**	**-0.52*†‡ (0.01)**	**-0.45*†‡ (0.02)**
Measures of glycemic variability (mg/dL)						
MAGE	0.07 (0.73)	-0.23 (0.25)	-0.21 (0.31)	-0.19 (0.35)	-0.23 (0.27)	-0.18 (0.39)
CONGA-1	0.06 (0.79)	-0.20 (0.32)	-0.17 (0.42)	-0.14 (0.50)	-0.20 (0.32)	-0.16 (0.44)
CONGA-2	0.07 (0.72)	-0.19 (0.35)	-0.17 (0.41)	-0.15 (0.48)	-0.19 (0.36)	-0.13 (0.51)
CONGA-4	0.04 (0.86)	-0.23 (0.26)	-0.18 (0.37)	-0.18 (0.39)	-0.19 (0.34)	-0.12 (0.54)
MODD	0.10 (0.62)	-0.14 (0.49)	-0.37 (0.07)	-0.27 (0.19)	-0.39 (0.06)	-0.32 (0.11)
Oxidative stress biological markers						

Nitric oxide (μmol/L)	0.14 (0.50)	-0.13 (0.50)	-0.32 (0.10)	-0.21 (0.28)	-0.27 (0.17)	-0.26 (0.19)
Myeloperoxidase (ng/mL)	-0.10 (0.63)	0.18 (0.38)	0.16 (0.41)	0.18 (0.37)	0.39†‡ (0.05)	0.31†‡ (0.11)
Oxidative stress ratio (μmol/L:ng/mL)	0.26 (0.18)	-0.23 (0.25)	-0.31 (0.11)	-0.32 (0.10)	**-0.39 (0.04)**	-0.29 (0.14)

Data represented as correlation coefficient (p value) with bolded entries corresponding to significant values ($p<0.05$)
PA=physical activity, LPA=light-intensity physical activity, MVPA=moderate-to-vigorous-intensity physical activity, LPA EE=light-intensity physical activity energy expenditure, MVPA EE=moderate-to-vigorous-intensity physical activity energy expenditure, METs=metabolic equivalents
*$p<0.05$ following adjustment for age; †$p<0.05$ following adjustment for BMI; ‡$p<0.05$ following adjustment for age and BMI

Table 5.4. Pearson product correlations between glucose concentrations and measures of glycemic variability with biological markers of oxidative stress

	Oxidative stress biological markers		
	Nitric oxide (μmol/L)	Myeloperoxidase (ng/mL)	Oxidative stress ratio (μmol/L:ng/mL)
Glucose concentrations (mg/dL)			
Fasting	**0.65***†§ **(<0.001)**	**-0.41***†‡§ **(0.03)**	**0.60***†‡§ **(<0.001)**
24-hour mean	0.17 (0.39)	-0.32 (0.11)	0.33 (0.09)
Diurnal	0.19 (0.34)	-0.23 (0.25)	0.28 (0.16)
Nocturnal	0.10 (0.63)	-0.34 (0.09)	0.30 (0.13)
Measures of glycemic variability (mg/dL)			
MAGE	0.14 (0.48)	**-0.46***†‡§ **(0.02)**	0.34*†‡§ (0.08)
CONGA-1	0.10 (0.61)	-0.35 (0.08)	0.28†‡ (0.16)
CONGA-2	0.08 (0.66)	**-0.39**§ **(0.04)**	0.27*† (0.18)
CONGA-4	0.15 (0.46)	**-0.38**†‡§ **(0.04)**	0.30 (0.13)
MODD	0.26 (0.21)	-0.29 (0.15)	0.32 (0.11)

Data represented as correlation coefficient (p value) with bolded entries corresponding to significant values ($p<0.05$)
$^{*}p<0.05$ following adjustment for age; $^{\dagger}p<0.05$ following adjustment for BMI; $^{\ddagger}p<0.05$ following adjustment for age and BMI; $^{\S}p<0.05$ following adjustment for time spent sedentary and performing LPA, MVPA, and total PA

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CHAPTER 6

MANUSCRIPT 3-EFFECT OF A 12-WEEK AEROBIC EXERCISE INTERVENTION ON GLYCEMIC VARIABILITY, VASCULAR HEALTH, AND OXIDATIVE STRESS IN OVERWEIGHT OR OBESE ADULTS-A PILOT STUDY[3]

[3]Sparks, JR, Sarzynski, MA, Davis, JM, Grandjean, PW, & Wang, X. To be submitted to *Journal of Physical Activity and Health*

ABSTRACT

Introduction: The state of being overweight or obese leads to an increased risk of development of cardiometabolic disease. Increases in glycemic variability have been associated with greater induction of oxidative stress and decreased vascular health, which may be exacerbated by higher weight status and improved through exercise. The purpose was to examine the impact of a 12-week aerobic exercise intervention on glycemic variability, vascular health, and oxidative stress in overweight or obese adults.

Methods: Eight overweight or obese adults (BMI=29.4±8.3 kg/m^2; age=48.9±5.2 years; mean±SD) completed a 12-week aerobic exercise intervention. Participants walked 3 times per week at moderate intensity and expended 10-12 kilocalories per kilogram of body weight each week. All participants wore a continuous glucose monitor (CGM) and completed a daily dietary intake diary for 7 consecutive days at baseline and post-intervention. On the final day of monitoring, a fasting blood sample was collected and an oral glucose tolerance test (OGTT) was performed. Intra- and inter-day glycemic variability was assessed as the mean amplitude of glycemic excursions, continuous overlapping net glycemic action of 1-, 2-, and 4-hour, and the mean observation of daily differences. Vascular health and oxidative stress markers, plasma concentrations of nitric oxide and myeloperoxidase, were measured, and their ratio was calculated.

Results: No OGTT glucose concentrations or measures of glycemic variability changed from baseline to post-intervention. However, myeloperoxidase concentration decreased (24.8±8.2 ng/mL to 16.4±4.6 ng/mL, $p<0.01$) and the ratio of nitric oxide concentration to myeloperoxidase concentration improved (3.5:1 to 6.4:1, $p<0.01$), while average daily

mealtime was delayed (13:30±00:40 hours to 14:00±00:39 hours, p=0.02) following the 12-week intervention.

Conclusion: Twelve weeks of aerobic exercise reduced oxidative stress and improved the ratio of nitric oxide concentration to myeloperoxidase concentration, but not glucose concentrations or glycemic variability in this group of overweight or obese non-diabetic adults.

INTRODUCTION

The prevalence of adults in the United States who are considered overweight or obese continues to rise with ~40% of the population designated obese in 2015-2016 (Centers for Disease Control and Prevention, 2017). Obesity is widely considered a major public health crisis of the current generation (Finkelstein EA, Trogdon JG, Cohen JW, & Dietz, W, 2009; Flegal MF, Kruszon-Moran D, Carroll MD, Fryar CD, & Ogden CL, 2016; Centers for Disease Control and Prevention, 2017). Overweight and obesity-related cardiometabolic disorders lead to an increased medical care cost, such as for treatment of impaired glycemic health and diagnosed cardiovascular disease (CVD) risk factors (Bray GA, 2004; O'donovan G, Kearney EM, Nevill AM, Woolf-May K, & Bird SR, 2005).

Previous findings from a large community-based cohort study found that body mass index (BMI) positively correlated with circulating biological markers of oxidative stress (Keaney Jr JF, Larson MG, Vasan RS, Wilson PWF, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, & Benjamin EJ, 2003). Oxidative stress is preceded by an increased exposure to reactive oxygen species, and damage to proteins, nucleic acids, and cell membranes, and has been implicated in the pathogenesis related to increased CVD risk factors, such as impaired vascular health, and subsequent CVD (Witztum JL & Steinberg D, 1991; Ohara Y, Peterson TE, & Harrison DG, 1993; Stortz G & Imlay JA, 1999; Cooke J 2004). Previously, oxidative stress was believed to be a key regulator in the development of diabetic complications (Baynes JW, 1991); however, evidence has suggested that an increase in oxidative stress is triggered by exacerbated oscillations in glucose concentrations (Hirsch IB & Brownlee M, 2005; Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, & Colette C, 2006). These exacerbated glucose

oscillations, known as glycemic variability, act to induce stress on the vascular endothelium and elicits endothelium-derived micro and macrovascular complications, which potentially increases CVD risk (Johnson EL 2013). In fact, the interaction between increased glycemic variability and elevated oxidative stress has recently been proposed as a mechanism for increased CVD risk and CVD progression in adults with and without diagnosed diabetes (Saisho Y 2014; Gorst C, Kwok CS, Aslam S, Buchan L, Kontopantelis E, Myint PK, Heatlie G, Loke Y, Rutter MK, & Marna MA, 2015).

Greater glycemic variability, impaired vascular health, and increased oxidative stress have both been observed independently in overweight or obese adults in the presence and absence of diagnosed diabetes or CVD (Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer X, & Eckel RH, 2006; Vincent HK, Innes KE, & Vincent KR, 2007; Fysekidis M, Cosson E, Banu I, Duteil C, & Valensi P, 2014). Exercise is often utilized as a therapeutic treatment for overweight or obesity related insulin resistance and cardiovascular strain (Goodyear LJ & Kahn BB, 1998; Hawley JA, 2004; Church T, 2011). Exercise training in overweight or obese adults in the presence or absence of diabetes, elicits beneficial alterations in fasting glucose concentrations, glucose tolerance during an oral glucose tolerance test (OGTT), and glucose regulation during glycemic clamp procedures independent of changes in body weight or cardiorespiratory fitness (CRF) (Swartz AM, Strath SJ, Bassett Jr DR, Moore JB, Redwine BA, Groër M, & Thompson DL, 2003; Cox KL, Burke V, Morton AR, Beilin LJ, & Puddey IB, 2004; King DS, Dalsky GP, Clutter WE, Young DA, Staten MA, Cryer PE, Holloszy JO 1988). Yet, past studies have been largely limited to laboratory-based assessment of glucose metabolism and tolerance. Recent advancements in continuous

glucose monitor (CGM) technology allows for the inclusion of a free-living condition, which enables further determination as to how exercise influences glucose concentrations and glycemic variability throughout the day.

Using CGM, Mikus et al. (2012), found that the frequency, magnitude, and duration of the number of glycemic excursions each day were decreased over the final 3 days of a 7-day aerobic exercise training program compared to 3 days of performing habitual exercise in non-insulin dependent type 2 diabetes mellitus patients (Mikus CR, Oberlin DJ, Libla J, Boyle LJ, & Thyfault JP, 2012). Another study by Farabi et al. (2015) found that a single 30-minute bout of aerobic exercise reduced diurnal glycemic variability and urinary isoprostanes, a biological marker of oxidative stress, in obese adults diagnosed with type 2 diabetes mellitus or impaired glucose tolerance (Farabi SS, Carley DW, Smith D, & Quinn L, 2015). Thus, the majority of research examining glycemic variability and oxidative stress has focused on diabetic populations with even fewer studies examining changes due to exercise training (Tereda T, Friesen A, Chahal BS, Bell GJ, McCargar LJ, & Boulé NG, 2013; Van Dijk J-W, Manders RJ, Canfora EE, van Mechelen WV, Hartgens F, Stehouwer CD & van Loon LJC, 2015).

Overweight or obese adults without diabetes may already have greater glycemic variability and oxidative stress, and impaired vascular health, but it is unknown whether they can be changed by exercise training in this population. Therefore, the primary purpose of this study was to examine glucose concentrations and glycemic variability using CGM in overweight or obese non-diabetic adults undergoing 12-weeks of moderate-intensity aerobic exercise. A secondary purpose was to evaluate the effects of the aerobic exercise intervention on biological markers of vascular health, nitric oxide, a

potent vasodilator, and oxidative stress, myeloperoxidase, a potent vasoconstrictor. We also examined changes in body weight, CRF, and dietary intake following the aerobic exercise intervention, as changes in these measures may influence outcomes of interest (Gaesser GA, Tucker JW, Jarrett CL, Catherine L, & Angadi SS, 2015; Pronk NP, 2015).

METHODS

Design Overview

The Aerobic Treadmill Exercise and Metabolism (A-TEAM; NCT: 03162991) study openly recruited from October 2017 to December 2018. The study protocol was approved by the University of South Carolina Institutional Review Board and all participants signed an informed consent form prior to participation. All participant visits, testing, and exercise sessions were completed and supervised by the same trained research staff and took place in the Clinical Exercise Research Center (CERC) housed within the Norman J. Arnold School of Public Health at the University of South Carolina.

Participants

Participants were not physically active (<120 minutes of resistance or moderate-intensity endurance exercise per week during the previous 3 months), overweight or obese ($25 \leq BMI \leq 40$ kg/m^2) males and females, age 35-55 years, weight stable (±2%) during the previous 3 months, and, for females, eumenorrheic. Exclusion criteria included any self-reported medical conditions such as diabetes or taking medications that are known to affect metabolism (e.g. statins), CVD, chronic or recurrent respiratory conditions, including uncontrolled asthma or chronic obstructive pulmonary disease, or active cancer. Individuals self-reporting diagnosed eating or neurological disorders, and psychological issues, including but not limited to untreated depression and attention

deficit disorder were also excluded. Additionally, excessive caffeine use (>500 mg/day), smoking during the past year, pregnant or lactating females, and/or unwillingness to provide informed consent were other reasons for exclusion.

A total of 64 participants were initially screened by telephone (Figure 6.1.). Ten participants began the exercise intervention, with 8 completing the 12-week exercise intervention. The 2 participants who dropped-out during the intervention completed 1 and 11, or ~3% and 30%, of the intervention visits, respectively. These participants did not provide specific reasons why they dropped-out and discontinued the study, as they ceased correspondence and were unable to be contacted to reschedule their intervention visits. Therefore, the 8 participants that completed the intervention were included in this analysis.

Intervention

Participants walked on treadmills 3 times per week in the CERC under supervision. Each week, participants had their body weight measured, which was utilized to calculate weekly exercise volume by multiplying each participants' body weight by 10-12 kilocalorie per kilogram of body weight each week (KKW). The frequency of the intervention visits was established as increases in exercise frequency has previously been shown to be a determinant of physiological responses to exercise training once 3 times per week have been achieved (Shephard RJ, 1968). As the participants in our study were not engaging in structured physical activity prior to the exercise intervention, 3 times per week was also chosen to limit exercise-related overuse injuries. The exercise volume was achieved by varying the duration, speed, and grade to reach each participants' weekly energy expenditure goal while maintaining moderate-intensity treadmill walking, which

was closely monitored throughout the 12-week intervention. Twelve weeks of aerobic exercise training has been previously studied and is considered a duration that physiological adaptations to aerobic exercise tend to occur (Ho SS, Dhaliwal SS, Hills AP, & Pal S, 2012). The prescribed training volume, 10-12 KKW, is a higher volume of aerobic exercise energy expenditure compared to current physical activity guidelines, which equates to 8 KKW (Morss GM, Jordan AN, Skinner JS, Dunn AL, Church TS, Earnest CP, Kampert JB, Jurca R, & Blair SN, 2004; Sisson SB, Katzmarzyk, Earnest CP, Bouchard C, Blair SN, & Church TS, 2009; Rosenkilde M, Reichkendler MH, Auerbach P, Bonne TC, Sjödin A, Ploug T, & Stallknecht BM, 2015; Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, George SM, & Olson RD, 2018). To monitor adherence to the exercise prescription, the energy expenditure of each exercise session was calculated using the American College of Sports Medicine formula: {0.1×(speed[miles per hour] ×26.8)+1.8×(speed[miles per hour] ×26.8)×grade(%)+3.5}×body weight(kg)÷5(L per minute)×time(minutes) (American College of Sports Medicine, 2017).

All exercise intervention visits were completed and supervised by trained research staff members at the University of South Carolina. Due to the physically inactive state of the participants, the exercise intensity and weekly energy expenditure were gradually increased to reduce risk of injury. Training intensity increased during the first 4 weeks of the exercise intervention until the target level of 50-55% of participant's heart rate reserve (HRR) was met, calculated as {[(peak heart rate-resting heart rate)×intensity (50-55%)]+resting heart rate}, which was determined during the baseline graded exercise test. Participants began at a weekly energy expenditure of 6-8 KKW during the first week

of the intervention and then progressed until week 4 when they attained their weekly energy expenditure of 10-12 KKW. Each exercise session began and ended with a 3-minute warm-up and cool-down. Heart rate (HR) monitors (FT1; Polar, Lake Success, NY, USA) were worn to monitor exercise intensity continuously throughout each exercise session and HR was recorded every five minutes. If HR monitors were unable to detect HR, manual palpation at the radial artery was measured for 30-60 seconds. Blood pressure was measured before, during warm-up, at the mid-point of the exercise session, during cool-down, and following each exercise session.

Compliance to prescribed exercise intervention (frequency, intensity, and duration) for each participant was reviewed weekly and any participant missing an exercise session without notifying study personnel was contacted via phone or e-mail to reschedule and encourage further attendance.

Measurements and Testing

Height, Body weight, and Body Mass Index (BMI)

Height and body weight were measured at the first baseline and post-intervention visit using a stadiometer and an electronic scale that was calibrated annually (CC Vaughan & Sons, Incorporated, Columbia, SC). Additionally, body weight was collected weekly throughout the 12-week intervention. BMI was calculated at baseline and post-intervention utilizing their respective height and body weight and the following equation: $BMI(kg/m^2)=Body\ Weight(kg)\div[Height(m)]^2$.

Graded Exercise Test

All participants performed a maximal graded exercise test on a treadmill at baseline and post-intervention. A ramped medium protocol was determined to be ideal

for these participants as it is an incremental protocol where speed and grade increase linearly every 30-60 seconds until each participant reached volitional fatigue (Hsia D, Casburi R, Pradhan A, Torres E, & Porszasz J, 2009). Volume of oxygen consumed ($\dot{V}O_2$) via metabolic cart (TrueOne 2400, ParvoMedics, Salt Lake City, UT) and HR using standard 12-lead electrocardiogram (Q-Stress®; Cardiac Science, Bothell, WA, USA) were monitored continuously during the progression of the test. Blood pressure was measured, and rating of perceived exertion was obtained every two-minutes during the test. Two of four generally recognizable criteria for the test to be considered satisfactory needed to be achieved: a respiratory exchange ratio ≥1.10; a rating of perceived exertion ≥17 on the Borg scale ranging from 6-20; achieving a maximum heart rate >90% age-predicted maximum HR (220-age); and/or a plateau in absolute $\dot{V}O_2$ ≤150 ml/min with an increase in exercise intensity. Relative peak oxygen consumption ($\dot{V}O_{2peak}$; ml/kg/min) was determined by the highest 30-second average $\dot{V}O_2$ value measured during the test. All graded exercise tests took place after 12:00 hours and minimum of 24 hours after the final day of the CGM monitoring period.

CGM Placement, Instruction, and Monitoring

A Dexcom G4 Platinum Professional CGM device (San Diego, CA, USA) was used to assess interstitial glucose concentrations over 7 consecutive days at baseline and post-intervention. At the first baseline and post-intervention visit participants reported to the CERC for placement and instruction of use for the CGM device by trained research staff. At post-intervention, the CGM device was placed a minimum of 72 hours after the last exercise session to limit to influence of the last exercise session on glucose concentrations. Participants had a catheter inserted under the skin, approximately 2 cm to

the side of the umbilicus, on the preferred side of the abdomen with an attached sensor and transmitter. They were instructed to carry a recording device which received and stored interstitial glucose concentration readings every 5 minutes over the 7 consecutive days. This specific CGM device model requires participants to manually perform a capillary blood measurement by fingerstick using a provided glucometer twice a day during days of wear per manufacture instructions. Participants were instructed on how to perform a capillary blood measurement and enter the glucometer readings into the CGM device.

The Dexcom G4 Platinum Professional CGM device has been validated and proven accurate against directly evaluated blood glucose concentrations (Facchinetti A, Favero SD, Sparacubi G, & Cobelli C, 2015). The CGM device was blinded so that participants could not observe the live readings to deter any alterations in diet, physical activity, or general lifestyle, and participants were requested to maintain their normal daily routine during the 7-day monitoring period at baseline and post-intervention. On the final day of the 7-day monitoring period, participants reported back to the CERC to complete a 2-hour OGTT and have the device removed. Data were considered valid for analysis if data were obtained by the device for at least 5 days including at least one weekend day, with a minimum available glucose measure over 20 hours, which equated to a minimum 240 out of a possible 288 glucose readings each day. Software provided by the manufacturer (Dexcom Studio 12.0.4.6) was used to download and export CGM data to Excel datafiles.

Oral Glucose Tolerance Test (OGTT)

The OGTT was conducted following an overnight fast (~12 hours other than water), participants reported to the CERC and had a venous blood sample collected. Time was recorded and matched with the CGM to establish fasting glucose time point. Following the venous blood collection, participants were instructed to consume a standard 10-ounce, 75-gram glucose infused drink (Azer Scienmtific, Morgantown, PA) within 5 minutes and the time consumption completed was recorded. Every 30-minutes afterwards, time was recorded until 2 hours were complete (Fasting, 30-, 60-, 90-, and 120-minutes post-consumption), which is the standard procedure for a 75-gram OGTT (Institute for Quality and Efficiency in Healthcare, 2011). During the OGTT, participants were instructed to limit their physical movement, unless necessary, to lessen the impact of muscle contraction on glucose concentrations. Glucose concentrations during the OGTT were determined by the CGM device rather than from blood samples. If any of the time points fell between CGM glucose concentration readings, as the CGM assesses glucose concentrations every 5 minutes, the average between the previous and following CGM concentration readings were calculated. The CGM-assessed glucose concentrations have been validated with venous blood glucose concentrations (Kovatchev B, Anderson S, Heinemann L, & Clarke W, 2008). OGTT AUC was calculated utilizing the equation Glucose AUC=$1/2\times30\times(y_{Fasting}+2y_{30}+2y_{60}+2y_{90}+y_{120})$, where y represents glucose concentration at the different time points (Tai MM, 1994). All OGTTs took place between 06:00 and 09:00 HH:MM and at least 72 hours after the last bout of aerobic exercise at post-intervention.

Daily Dietary Intake

Daily dietary intake was recorded at baseline and post-intervention during the same 7 consecutive days as the CGM monitoring and all participants were instructed to continue their normal daily dietary routine during this time. All participants self-reported calorie consumption, including any calorie containing beverages or snacks and time of consumption for standard meals (breakfast, lunch, dinner, and snacks). In order to do this, they were instructed on basic measurement of portion sizes to aid in recording of each meal using the MyFitnessPal application (MyFitnessPal, Inc.) that is available on smartphone devices or computer based. If a smartphone or computer was unavailable, participants were provided a self-report paper form to record any calorie containing food or drink consumed, including portion size, calories, and macronutrient breakdown (carbohydrate, protein, and fat) utilizing The Calorie King® Calorie Counter (Borushek A, 2015). Calorie intake, macronutrient breakdown, and time of consumption were calculated for each standard meal and the total of each day's standard meals was calculated for each valid recording day, which was analyzed as an average of those valid days. Additionally, the standard deviation (SD) of daily dietary intake across all valid days were calculated as a measure of day-to-day variability.

Previously, habitual dietary intake has been related to fasting and OGTT glucose concentrations, as well as biological markers of oxidative stress (Feskens EJ & Kromhout D, 1990; Kleemola P, Freese R, Jauhiainen M, Pahlman R, Alfthan G, & Mutanen M, 2002). Therefore, potential changes in habitual dietary intake and their influence on glucose concentrations, glycemic variability, and oxidative stress need to be considered.

Glucose Concentrations

Twenty-four-hour mean, diurnal, nocturnal, pre- and post-waking, and post-meal glucose concentrations were calculated for each valid day and expressed as the average across those days. 24-hour mean glucose concentration was assessed from midnight to midnight for each valid day. Diurnal and nocturnal glucose concentrations were assessed each valid day during each participants' self-reported time-in-bed and time out of bed. Further, analysis of exploratory measures of glucose metabolism were performed for pre-waking, waking, post-waking, and post-meal glucose concentrations.

Pre- and post-waking time-point glucose concentrations were established as 1-, 2-, and 3-hour time points prior to and after waking time-point, while pre- and post-waking average glucose concentrations were established as the average of over 1-, 2-, and 3-hour periods prior to and after waking time-point. These time points and periods were chosen as they potentially relate to episodes of nocturnal hypoglycemia and subsequent risk of rebound hyperglycemia, also described as the Somogyi effect, previously observed in insulin-treated diabetic patients (Campbell IW, 1976; Gale EA, Kurtz AB, & Tattersall RB, 1980). After awakening, none of the participants consumed any calorie containing food or drink items within the 3-hour post-waking timeframe on each valid day utilized. However, most participants reported coffee (caffeine) consumption within 2 hours post-waking, with none reporting calorie containing additive, such as sugar, milk, or creamer.

Post-meal glucose concentrations were assessed over a 2-hour time period following subjectively recorded time of consumption for each participants' breakfast, lunch, dinner, and snacks, as well as the average of all meals combined for total daily dietary consumption. In the same fashion as the OGTT, glucose concentrations at time

points 0-, 30-, 60-, 90-, and 120-minutes were determined and AUC was calculated for each valid day, with the average across those days utilized for analysis.

Glycemic Variability

The continuous overlapping net glycemic action of 1-hour, 2-hour, and 4-hour (CONGA-1, CONGA-2, and CONGA-4) was calculated in Excel. CONGA-1, CONGA-2, and CONGA-4 were calculated as the standard deviation of the difference between each observation and the previous 1-hour, 2-hour, and 4-hour observations (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005; Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, & Heine RJ, 2008; Kuenen JC, Borg R, Zheng H, Schoenfeld D, Heine RJ, & Nathan DM, 2011). CONGA-1, CONGA-2, and CONGA-4 were chosen for this study because the 1-hour, 2-hour, and 4-hour time periods approximates the time intervals between different activities (CONGA-1), time between snacks (CONGA-2), and time between meals (CONGA-4), respectively (McDonnell CM, Donath SM, Vidmar SI, Werther GA, & Cameron FJ, 2005). To calculate the mean amplitude of glycemic excursion (MAGE) and mean of daily differences (MODD) measures of glycemic variability the Excel data were transferred into the EasyGV Version 9.0.R2 (University of Oxford, Oxford, England, UK), which is an Excel-enabled workbook that utilizes macros. MAGE and CONGA-1, CONGA-2, and CONGA-4 were measurements of intra-day glycemic variability for each valid day of wear time and averages of those days calculated. MAGE was calculated for each subject by taking the arithmetic mean of increased or decreased glucose concentrations (nadirs and peaks or vice-versa) when both ascending and descending concentration exceeds one standard deviation for the same 24-hour monitoring period (Service FJ, Molnar GD, Rosevear JW,

Ackerman E, Gatewood LC, & Taylor WF, 1970; Service FJ & Nelson RJ, 1980; Service FJ, O'Brien PC, & Rizza RA, 1987). MODD served as a measurement of inter-day glycemic variability, which was calculated for each two consecutive day period and averaged to include all valid days over the seven-day monitoring period. MODD accounts for the mean of absolute differences between glucose concentrations obtained at the same time of day on consecutive days (Rodbard D, Matsubara B, Nakamura K, Bailey T, Jovanovic L, Zisser H, Kaplan R, & Garg SR, 2009).

Biological Markers of Vascular Health and Oxidative Stress

Fasting venous blood samples were collected and used to determine biological markers of vascular health and oxidative stress. Blood samples were collected into a BD Vacutainer EDTA plasma collection tube. Immediately following collection, blood samples were centrifuged at 3000 rpm and 4ºC for 20 minutes immediately following collection. Plasma separated after centrifugation were aliquoted into 1.5mL centrifugation tubes and stored at -80ºC until all participant's samples were ready for analysis. Prior to analysis, plasma samples were thawed and re-centrifuged at 3000 rpm and 4ºC for 20 minutes to ensure separation of any particulate.

Nitric oxide, which acts as a potent vasodilator and measure of vascular health (Palmer RMJ, Ashton DS, & Moncada S, 1988), and myeloperoxidase, a potent vasoconstrictor and measure of oxidative stress (Klebanoff SJ, 2005), were measured using two separate enzyme-linked immunoabsorbant assays (ELISA). These biological markers of vascular health and oxidative stress were chosen for this study as they have previously been observed to be related to measures of glycemic health in adults diagnosed with type 2 diabetes mellitus (Kingwell BA, Formosa M, Muhlmann M,

Bradley SJ, & McConell GK, 2002; Unubol M, Yavasoglu I, Kacar E, Omurlu IK, Ture M, Kadikoylu G, & Bolaman Z, 2015).

The nitric oxide ELISA kit (ThermoFicher Scientific, Waltham, MA) quantitatively determines the concentrations of nitrate and nitrite in plasma samples. This ELISA utilizes the enzyme nitrate reductase to convert nitrate to nitrite, which was then detected as a colored azo dye product of the Griess reaction which absorbs light at 540 nm. The interaction of nitrate and nitrite concentrations determine the concentration of nitric oxide in plasma (Fareed D, Tqbal O, Tobu M, Hoppensteadt DA, & Fareed J, 2004). The myeloperoxidase ELISA kit (Eagle Biosciences, Inc., Nashua, NH) quantifies myeloperoxidase utilizing a two-site "sandwich" technique that binds to different epitopes of myeloperoxidase. After several incubation periods and plate washed, antibodies bound to myeloperoxidase were ready to analyze by detecting the immunocomplex and the absorbency of the sample set at 405 nm with the reference filter set at 620-650 nm. The ratio or nitric oxide concentration to myeloperoxidase concentration was calculated by the concentration of nitric oxide divided by the concentration of myeloperoxidase to examine balance between measures of vasodilation and vasoconstriction. Proper sample dilution and preparation per manufacture product information sheet were followed. All assays were performed and analyzed on the same day by the same trained research staff with an intra-assay variability of <8%.

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 (Cary, NC) for the 8 participants to complete the intervention. Participant characteristics were calculated and reported as mean and standard deviation (Mean±SD). All outcome variables of interest

were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Change values were calculated by subtracting baseline values from post-intervention values. Paired sample t-tests were performed to determine if any values significantly changed from baseline to post-intervention. A general linear model was utilized to adjust for change in body weight and/or change in relative $\dot{V}O_{2peak}$ for primary outcomes of interest, including glucose concentrations, glycemic variability measures, and biological markers of vascular health and oxidative stress. Pearson product correlations were performed between changes in glucose concentrations and measures of glycemic variability with changes in biological markers of vascular health and oxidative stress to determine the association between these primary outcomes of interest. Lastly, as change in body weight or $\dot{V}O_{2peak}$ may influence our outcomes of interest, they were adjusted for in the analyses. A p value <0.05 was considered statistically significant.

RESULTS

Participant Characteristics

Participant characteristics are included in table 6.1. The participants were overweight or obese, middle-aged adults, with an even distribution between males and females. The overall relative $\dot{V}O_{2peak}$ (27.7±9.0 ml/kg/min) and relative $\dot{V}O_{2peak}$ for both men (31.1±10.9 ml/kg/min) and women (24.4±6.2 ml/kg/min) was determined to be lower than the standard normal values for their age and sex and thus reflective of being sedentary prior to the intervention (Myers J, Kaminsky LA, Lima R, Christle JW, Ashley E, & Arena R, 2017).

Exercise Intervention Effects

Data on the prescribed and actual completed exercise for participants that completed the exercise intervention are included in table 6.2. The exercise intensity (50-55% HRR), volume (10-12 kcal/kg/week), and number of sessions (36-37) were set standards throughout the intervention. If participants missed ≥3 exercise sessions, additional sessions were included to make-up for the missed sessions. All participants walked 120-135 minutes per week (128.8±4.4 minutes per week).

Body Weight, BMI, and Graded Exercise Test

Body weight, BMI, and graded exercise test measurements obtained at baseline and post-intervention are included in table 6.3. Overall, there were no significant changes in body weight and BMI. Further, all participants met two of four generally recognizable criteria needed to be achieved for the graded exercise test to be considered satisfactory at baseline and post-intervention. Relative $\dot{V}O_{2peak}$, and maximal respiratory quotient, heart rate, and blood pressure, as well as rating of perceived exertion did not change significantly from baseline to post-intervention. Treadmill time, however, significantly increased indicating improved exercise tolerance and performance during maximal exercise. Additionally, individual changes from baseline to post-intervention for body weight and $\dot{V}O_{2peak}$ were included (Figures 6.2. and 6.3., respectively). Although the data were normally distributed at baseline and post-intervention, one participant had a noticeably greater decrease in body weight (participant 6), while another participant had a noticeably greater increase in $\dot{V}O_{2peak}$ (participant 2).

Glucose Concentrations and Glycemic Variability Assessed by CGM

Glucose concentrations and measures of glycemic variability at baseline and post-intervention are included in table 6.4. There were no significant changes in fasting and glucose concentrations during OGTT, and OGTT AUC from baseline to post-intervention. Also, there was not a significant difference from baseline to post-intervention for 24-hour mean, diurnal, or nocturnal glucose concentrations. Further, no measure of intra-day glycemic variability, MAGE, CONGA-1, CONGA-2, CONGA-4, or inter-day glycemic variability, MODD, significantly changed from baseline to post-intervention. Following adjustment for body weight and/or relative $\dot{V}O_{2peak}$, these findings persisted as all CGM-assessed glucose concentrations and glycemic variability measures did not significantly change from baseline to post-intervention ($p \geq 0.05$ for all).

Vascular Health and Oxidative Stress

Biological markers of vascular health and oxidative stress at baseline and post-intervention are included in table 6.4. There was a significant decrease in myeloperoxidase concentration and a significant increase in the ratio of nitric oxide concentration to myeloperoxidase concentration, with neither remaining significant following adjustment for change in body weight and/or relative $\dot{V}O_{2peak}$ ($p \geq 0.13$ for all).

Relationship between Change in Glucose Concentrations and Glycemic Variability with Change in Vascular Health and Oxidative Stress

The association between the change in glucose concentrations and glycemic variability with the change in vascular health and oxidative stress measurements are included in table 6.5. There were significant negative correlations found between change in diurnal glucose concentration with change in nitric oxide concentration, as well as

between change in nocturnal glucose concentration with change in myeloperoxidase concentration.

Following adjustment for change in body weight and/or change in relative $\dot{V}O_{2peak}$, change in nitric oxide no longer significantly correlated with change in diurnal glucose concentrations ($p=0.05$). However, following adjustment for change in body weight only, change in myeloperoxidase concentration continued to negatively correlate with change in nocturnal glucose concentration ($r=-0.97$, $p=0.03$), while change in nitric oxide concentration negatively correlated with change in nocturnal glucose concentration ($r=-0.97$, $p=0.03$). Interestingly, following adjustment for relative $\dot{V}O_{2peak}$ only, change in diurnal glucose concentration negatively correlated, while MAGE and CONGA-1 measures of glycemic variability positively correlated, with myeloperoxidase concentrations ($0.97 \leq$ absolute $r \leq 0.99$, $p \leq 0.03$ for all).

Relationship between Change in Body Weight and/or $\dot{V}O_{2peak}$ with Glucose Concentrations, Glycemic Variability, Vascular Health, and Oxidative Stress

Further analyses were performed to examine the relationship between change in body weight, relative $\dot{V}O_{2peak}$ (ml/kg/min), and absolute $\dot{V}O_{2peak}$ (ml/min) with change in glucose concentrations, glycemic variability, vascular health, and oxidative stress (Table 6.6). Change in body weight negatively correlated with change in nocturnal glucose concentration, while change in relative $\dot{V}O_{2peak}$ did not correlate with any glucose concentration, glycemic variability measure, or biological marker of oxidative stress. Further, change in absolute $\dot{V}O_{2peak}$ negatively correlated with change in nocturnal glucose concentration, as well as MAGE and CONGA-1 measures of glycemic variability.

Waking Status and Post-Meal Glucose Concentrations

Exploratory analysis for post-meal and pre-/post-waking glucose concentrations were performed and are included in table 6.7. There were no differences in post-meal glucose concentrations following breakfast, lunch, dinner, or snacks; therefore, the daily total post-meal glucose concentrations and AUC were examined. The majority of the post-meal glucose concentrations and AUC did not significantly change other than an observable increase in 30-minute post-meal glucose concentration ($p=0.03$), which was lost following adjustment for change in body weight and/or relative $\dot{V}O_2peak$ (≥ 0.07 for all). Similarly, there were no changes in pre-waking, waking, or post-waking glucose concentrations ($p \geq 0.25$ for all), which persisted following adjustment for change in body weight and/or relative $\dot{V}O_2peak$ ($p \geq 0.18$ for all).

Daily Dietary Intake

Further evaluation of changes daily dietary intake was analyzed and presented in table 6.8. The average total daily mealtime when combining all meals, which included breakfast, lunch, dinner, and snacks, was found to be significantly later following the intervention when compared to baseline. Similar results were found when examining the day-to-day variability for meal measurements, including no changes in variability in mealtime, calories consumed, or grams per day of carbohydrate, protein, and fat.

DISCUSSION

To our knowledge this is one of the first studies to examine the effect of chronic center-based aerobic exercise on glucose concentrations and glycemic variability assessed by CGM with the inclusion of biological markers of vascular health and oxidative stress in overweight or obese non-diabetic adults. The primary findings were that myeloperoxidase concentration significantly decreased while the ratio of nitric oxide

concentration to myeloperoxidase concentration, significantly increased, suggesting reduced oxidative stress and improved ability to vasodilate compared to vasoconstrict following the intervention. However, there were no observable changes in CGM-assessed glucose concentrations or glycemic variability. Yet, average total daily mealtime was found to be significantly later following the 12-week intervention.

Effect of Aerobic Exercise Intervention on Biological Markers of Vascular Health and Oxidative Stress

A purpose of our study was to examine the effect of chronic exercise on circulating concentrations of nitric oxide, a potent vasodilator and measure of vascular health, and myeloperoxidase, a potent vasoconstrictor and measure of oxidative stress. Our study found a significant decrease in myeloperoxidase concentration and a significant increase in the ratio of nitric oxide concentration to myeloperoxidase concentration. A previous study aimed to assess whether a 12-week endurance exercise program could reduce biological markers of oxidative stress in adults at an increased risk of coronary events and found that myeloperoxidase concentration significantly decreased after training (Richter B, Niessner A, Penka M, Grdić M, Steiner S, Strasser B, Ziegker S, Zorn G, Maurer G, Simeon-Rudolf V, Wojta J, & Huber K, 2005). Another study examined the effect of 16 weeks of aerobic exercise, which incorporated 2 different intensities (30-40% $\dot{V}O_{2max}$ or 55-65% $\dot{V}O_{2max}$) for 30 minutes each session, 3 times per week in sedentary, obese males that were non-diabetic (30-40% $\dot{V}O_{2max}$: n=6; 55-65% $\dot{V}O_{2max}$: n=6) or type 2 diabetics (30-40% $\dot{V}O_{2max}$: n=6; 55-65% $\dot{V}O_{2max}$: n=7) (Krause M, Rodrigues-Krause J, O'Hagan C, Medlow P, Davison G, Susta D, Boreham C, Newsholme P, O'Donnell M, Murphy C, & De Vito G, 2014). They found that skeletal muscle nitric oxide expression, but not circulating serum nitric oxide concentration,

increased following the 16 weeks of aerobic exercise at 55-65% $\dot{V}O_{2peak}$ in only non-diabetic participants, with no alterations in those performing lower-intensity aerobic exercise, or in adults diagnosed with type 2 diabetes following either exercise intensity. Therefore, our findings are in line with previous evidence that aerobic exercise training alters circulating biological markers of vascular health and oxidative stress, specifically decreasing myeloperoxidase concentration and increasing the potential to vasodilate.

Effect of Aerobic Exercise Intervention on CGM-Assessed Glucose Concentrations and Glycemic Variability

Unexpectedly, there were no significant changes in any glucose concentration or measure of glycemic variability from baseline to post-intervention. Mikus et al. (2012) examined the differences in glycemic variability during 3 days of habitual PA and during the final 3 days of a 7 day aerobic exercise program in obese adults diagnosed with type 2 diabetes mellitus that were not currently taking exogenous insulin (Mikus CR, Oberlin DJ, Libla J, Boyle LJ, & Thyfault JP, 2012). Their study found that 24-hour mean glucose concentration was not different during the final 3 days of aerobic exercise compared to the 3 days of habitual exercise. Further, a randomized control trial examined the effects of a 4-month free-living continuous walking or interval-walking training on CGM-assessed glycemic control in adults diagnosed with type 2 diabetes (Karstoft K, Winding K, Knudsen SH, Nielsen JS, Thomsen C, Pedersen BK, & Solomon TPJ, 2013). Their study found elevated 24-hour mean glucose concentration in the control group, who underwent no exercise, while 24-hour mean glucose concentration decreased in the interval-walking group with no changes noted in the continuous-walking group. A more recent clinical trial in type 2 diabetics examined the effects of 12 weeks of low-volume high-intensity interval training (HIIT), including both aerobic- and resistance-based

exercise, on cardiometabolic outcomes (Francois ME, Durrer C, Pistawka KJ, Halperin FA, Chang C, & Little JP, 2017). Their study found significant decreases in 24-hour mean glucose concentration and MAGE following the 12 weeks of HIIT training. These studies provide evidence that 24-hour glucose concentration and glycemic variability are potentially lowered by acute and chronic exercise in type 2 diabetics. However, findings from previous studies examining the influence of exercise on glucose concentrations and glycemic variability are not in line with findings from our study in overweight or obese non-diabetic adults undergoing chronic exercise training.

Confounders to Our Findings

Reasons behind the absence of reductions in free-living glucose concentrations and glycemic variability in our study may be explained. Our study was designed for the CGM monitoring period to begin and subsequent fasting blood sample collection and OGTT to occur ≥72 hours after the last exercise session. This timeframe was determined in order to limit the acute effect of the last bout of exercise on glucose concentrations, glycemic variability, and oxidative stress, as even a single bout of exercise could influence our outcomes of interest **(**Mikines KJ, Sonne B, Farrell PA, Tronier B, & Galbo H, 1988; Radak Z, Chung HY, Koltai E, Taylor AW, & Goto S, 2008)**.** Although this timing was within the time frame before potential detraining effects on biological markers of oxidative stress occur (Maeda S, Miyauchi T, Kakiyama T, Sugawara J, Iemitsu M, Irukayama-Tombe Y, Murakami H, Kumagi Y, Kuno S, & Matsuda M, 2001), the effects of exercise on glucose homeostasis-related insulin sensitivity begins to be lost within 5-10 days following cessation of exercise training (Heath GW, Gavin III JR, Hinderliter JM, Hagberb JM, Bloomfield SA, & Holloszy JO, 1983). Therefore,

timing of CGM placement and subsequent first monitoring day may be a limiting factor as to why we observed no changes in glucose concentration or glycemic variability.

Further, previous evidence has suggested that alterations and potential compensation in dietary intake exists in adults following acute exercise and undergoing chronic aerobic exercise training (Church TS, Martin CK, Thompson AM, Earnest CP, Mikus CR, & Blair SN, 2009; Melzer K, Renaud A, Zurbuchen S, Tschopp C, Lehnman J, Malatesta D, Ruch N, Schutz Y, Kayser B, & Mäder U, 2016). This potential confounder may provide further insight into why we found no differences in glucose concentrations or glycemic variability as dietary caloric intake has been shown to influence glucose metabolism (Young LL 1984). Moreover, previous evidence has suggested that meal-timing potentially influences glucose metabolism (Sensi S & Capani F, 1987). In our study, average total daily mealtime was significantly later at post-intervention compared to baseline. A recent study examined the impact of a shift in mealtime at lunch for a week on postprandial glucose metabolism and glucose tolerance in healthy young women (Bandín C, Scheer FAJL, Luque, Ávila-Gandía V, Zamora S, Madrid JA, Gómez-Abellán P, & Garaulet M, 2015). Their study found that glucose concentrations were higher at 90- and 120-minutes post-meal consumption, and total post-meal glucose AUC was greater following consumption of a standardized lunch later in the afternoon (16:30 hours) compared to early afternoon (13:30 hours). The authors concluded that a shift in mealtime negatively influenced glucose tolerance in healthy young women. Although, we did not find any differences in the calorie amount or timing of each individual meal (i.e. breakfast, lunch, and dinner) or their respective glucose

concentrations following specific meals, the overall later mealtime may aid in the explanation of our findings.

Strengths and Limitations

The primary strength of this study was that all exercise sessions were monitored by trained research staff in the CERC, which controlled for the structure and environment of the exercise intervention. Another strength was the use of CGM to assess free-living glucose concentrations and glycemic variability for multiple days. Further, evaluation of changes in biological markers of vascular health and oxidative stress in conjunction with glycemic variability, while also exploring the relationship between changes in the primary outcomes of interest, were strengths of this study.

However, there exist several limitations. The primary limitation was the small sample size (n=8). Yet, there were significant changes observed in myeloperoxidase concentration and the ratio of nitric oxide concentration to myeloperoxidase concentration. Our study was initially designed to serve as a pilot and feasibility study to use CGM to assess glucose concentrations and glycemic variability in exercise training studies. We successfully used CGM in our study; however, the timing of placement of the CGM and collection of fasting blood samples may have influenced our findings. Even though we were attempting to limit the impact of the last bout of exercise on our outcomes of interest, we may have limited ourselves to evaluation of glucose concentrations during a de-training state as opposed to a trained state. Thus, placement of the CGM device earlier, even the same day as the last exercise bout, may provide greater insight into the effect of exercise training on free-living glucose concentrations and glycemic variability. Additionally, the exercise intensity (50-55% HRR), volume (10-12

KKW), or duration (12 weeks) may not have been sufficient to observe changes in clinical or free-living measures of glucose concentrations and glycemic variability. Lastly, the inclusion of a free-living condition where we did not test the results of a standardized diet on glucose concentrations was a potential limitation. Yet, this was also believed to be a strength, as not completely controlling for diet allows for a greater insight and potential dietary changes in adults incorporating exercise training into their daily life.

Conclusion

Although we found a significant decrease in myeloperoxidase concentration and an improvement in the ratio of nitric oxide concentration to myeloperoxidase concentration, there were no changes in any clinical-based or free-living glucose concentrations or measure of glycemic variability. This may partially be explained by an overall delay in total daily meal-timing from baseline to post-intervention, timing of placement and subsequent monitoring of CGM glucose concentrations and glycemic variability following exercise training, exercise intensity and volume during the intervention, as well as the overall duration of the intervention. Future studies should consider timing of the CGM device and a higher intensity of exercise to determine changes in glucose concentrations and glycemic variability in sedentary, overweight or obese adults undergoing aerobic exercise training.

Table 6.1. Participant baseline characteristics (n=8)

Variable	
Age (years)	48.9±5.2
Height (m)	1.7±0.1
Body weight (kg)	86.2±32.9
Body mass index (BMI; kg/m^2)	29.4±8.3
Sex (M/F)	4/4
Race (C/AA/AAA)	3/4/1
College graduate [n (%)]	6 (75)
Employed for wages [n (%)]	7 (87.5)
Income ≥$80,000.00 [n (%)]	4 (50)
Married [n (%)]	4 (50)
Number of children ≥1 [n (%)]	3 (37.5)

Data presented as Mean±SD or number (n) and percent (%) of participants
M=male, F=female, C=Caucasian, AA=African American,
AAA=Asian/Asian-American

Table 6.2. Exercise prescription and intervention adherence for participants who completed the study

	Intervention average
Exercise intensity	
Prescribed heart rate range (50 to 55% HRR, bpm)	124.9±8.8 to 129.2±9.1
Actual heart rate (bpm)	127.7±1.9
Exercise volume	
Prescribed exercise volume range (10 to 12 KKW)	843.6±292.6 to 1012.3±351.1
Actual exercise volume* (KKW)	863.8±225.6
Exercise compliance	
Prescribed number of exercise sessions‡	36 to 37
Actual number of exercise sessions	34.9±1.1

Data presented as Mean±SD
HRR=heart rate reserve; bpm=beats per minute; KKW=kilocalories per kilogram of body weight each week
*, exercise volume calculated using the American College of Sports Medicine formula (American College of Sports Medicine, 2017)
‡, 36 to 37 is dependent on inclusion of introductory exercise visit for exercise intervention

Table 6.3. Participant body weight, BMI, and graded exercise test measurements at baseline and post-intervention

	Baseline	Post-intervention	p value
Body weight (kg)	86.2±32.9	83.5±27.4	0.27
BMI (kg/m^2)	29.4±8.3	28.5±6.7	0.26
Graded exercise test measurements			
Relative $\dot{V}O_{2peak}$ (ml/kg/min)	27.7±9.0	29.6±8.5	0.21
Maximal respiratory quotient	1.2±0.1	1.2±0.2	1.00
Maximal rating of perceived exertion	18.3±1.1	18.3±0.9	0.83
Resting heart rate (bpm)	71.3±15.1	71.3±11.4	1.00
Resting systolic blood pressure (mmHg)	123.6±11.5	119.3±7.2	0.29
Resting diastolic blood pressure (mmHg)	79.3±7.5	79.9±7.5	0.82
Maximal heart rate (bpm)	174.0±15.5	176.0±15.5	0.49
Maximal systolic blood pressure (mmHg)	171.0±26.7	171.0±14.9	1.00
Maximal diastolic blood pressure (mmHg)	76.5±9.5	80.8±6.8	0.25
Treadmill time (minutes)	**10.6±2.7**	**11.8±2.3**	**0.04**

Data presented as Mean±SD
BMI=body mass index, $\dot{V}O_{2peak}$=peak volume of oxygen consumed
p values are for comparisons between baseline and post-intervention

Table 6.4. OGTT glucose concentrations, glycemic variability measures, and vascular health and oxidative stress biological markers at baseline and post-intervention

	Baseline	Post-intervention	p value
CGM observations per day (n; % of max)	271.3±11.2 (94.2%)	270.4±9.1 (93.9%)	0.92
Glucose concentrations			
Fasting (mg/dL)	88.1±9.0	92.9±11.3	0.38
30-minute (mg/dL)	125.2±28.8	138.4±33.8	0.31
60-minute (mg/dL)	139.1±25.6	137.6±29.2	0.92
90-minute (mg/dL)	126.5±19.5	130.7±19.7	0.73
120-minute (mg/dL)	105.3 (102.0-153.0)	117.0±18.0	0.95
OGTT AUC (mg/dL·2-hour)	14810.4±2334.8	15353.6±2603.5	0.69
24-hour mean (mg/dL)	100.5±13.5	103.4±9.7	0.29
Diurnal (mg/dL)	101.1±13.9	106.4±7.8	0.19
Nocturnal (mg/dL)	98.7±12.5	100.8±11.8	0.26
Measures of glycemic variability			
MAGE (mg/dL)	42.3 (36.8-60.9)	43.3±7.6	0.67
CONGA-1 (mg/dL)	18.7±3.9	20.3±3.4	0.56
CONGA-2 (mg/dL)	21.7±4.6	23.0±3.6	0.68
CONGA-4 (mg/dL)	22.9±4.8	25.4±3.3	0.34
MODD (mg/dL)	19.4±3.5	22.5±6.2	0.31
Vascular health and oxidative stress biological markers			
Nitric oxide (μmol/L)	83.4±23.8	102.9±33.7	0.09
Myeloperoxidase (ng/mL)	**24.8±8.2**	**16.4±4.6**	**<0.01**
Nitric oxide to myeloperoxidase ratio	**3.5±1.1**	**6.4±1.7**	**<0.01**

Data presented as Mean±SD, non-normally distributed data presented as median (lower quartile-upper quartile)

OGTT=oral glucose tolerance test, mg/dL=milligram per deciliter, AUC=area under the curve, MAGE=mean amplitude of glycemic excursion, CONGA-1, -2, and -4=continuous overlapping net glycemic action of 1-hour, 2-hour, and 4-hour, MODD=mean of daily differences

p values are for comparisons between baseline and post-intervention

Table 6.5. Pearson product correlations between change in glucose concentrations and measures of glycemic variability with change in biological markers of vascular health and oxidative stress

	Vascular health and oxidative stress biological markers		
	Nitric oxide	Myeloperoxidase	Nitric oxide to myeloperoxidase ratio
Glucose concentrations			
Fasting	-0.33 (0.42)	0.15 (0.73)	-0.46 (0.25)
120-minute	-0.09 (0.85)	-0.34 (0.46)	-0.06 (0.89)
24-hour mean	0.75 (0.08)	0.12 (0.82)	0.77 (0.08)
Diurnal	**-0.92 (0.01)**	-0.15‡ (0.78)	-0.64 (0.17)
Nocturnal	-0.02* (0.97)	**-0.98* (<0.001)**	0.36 (0.48)
Measures of glycemic variability			
MAGE	0.50 (0.32)	-0.47‡ (0.35)	0.69 (0.13)
CONGA-1	0.55 (0.26)	-0.42‡ (0.40)	0.72 (0.10)
CONGA-2	0.49 (0.32)	-0.32 (0.54)	0.59 (0.21)
CONGA-4	0.53 (0.28)	-0.05 (0.92)	0.51 (0.30)
MODD	0.29 (0.58)	-0.19 (0.72)	0.46 (0.36)

Data represented as correlation coefficient (p value) with bolded entries corresponding to significant values

MAGE=mean amplitude of glycemic excursion, CONGA-1, -2, and -4=continuous overlapping net glycemic action of 1-hour, 2-hour, and 4-hour, MODD=mean of daily differences

*, $p<0.05$ following adjustment for change in body weight; ‡, $p<0.05$ following adjustment for change in relative $\dot{V}O_{2peak}$

Table 6.6. Pearson product correlations between change in body weight, relative $\dot{V}O_{2peak}$, and absolute $\dot{V}O_{2peak}$ with change in glucose concentrations and measures of glycemic variability with change in biological markers of vascular health and oxidative stress

	Body weight	Relative $\dot{V}O_{2peak}$	Absolute $\dot{V}O_{2peak}$
Glucose concentrations			
Fasting	-0.02 (0.96)	0.16 (0.70)	-0.02 (0.97)
120-minute	-0.26 (0.58)	-0.16 (0.74)	0.10 (0.83)
24-hour mean	-0.00 (1.00)	-0.11 (0.84)	-0.03 (0.96)
Diurnal	0.29 (0.58)	0.23 (0.66)	0.19 (0.72)
Nocturnal	**-0.90 (0.02)**	0.00 (0.99)	**-0.86 (0.03)**
Measures of glycemic variability			
MAGE	-0.81 (0.05)	0.42 (0.40)	**-0.88 (0.02)**
CONGA-1	-0.78 (0.07)	0.37 (0.47)	**-0.83 (0.04)**
CONGA-2	-0.72 (0.11)	0.39 (0.44)	-0.76 (0.08)
CONGA-4	-0.52 (0.29)	0.44 (0.38)	-0.58 (0.23)
MODD	-0.48 (0.33)	0.46 (0.35)	-0.56 (0.25)
Vascular health and oxidative stress biological markers			
Nitric oxide	-0.01 (0.97)	-0.28 (0.50)	0.06 (0.88)
Myeloperoxidase	0.62 (0.10)	0.02 (0.96)	0.57 (0.14)
Oxidative stress ratio	0.02 (0.96)	-0.17 (0.69)	0.05 (0.90)

Data represented as correlation coefficient (p value) with bolded entries corresponding to significant values
MAGE=mean amplitude of glycemic excursion, CONGA-1, -2, and -4=continuous overlapping net glycemic action of 1-hour, 2-hour, and 4-hour, MODD=mean of daily differences

Table 6.7. Participant post-meal and wake status glucose concentrations at baseline and post-intervention

	Baseline	Post-intervention	p value
Post-meal glucose concentrations			
0-minute (mg/dL)	94.4±17.5	105.1±16.1	0.08
30-minute (mg/dL)	**99.7±16.5**	**113.0±12.8**	**0.03**
60-minute (mg/dL)	107.8±13.6	114.7±9.4	0.32
90-minute (mg/dL)	110.0±15.0	115.3±10.7	0.41
120-minute (mg/dL)	106.2±13.5	108.9±11.3	0.55
Post-meal AUC (mg/dL·2-hour)	12557.0±1542.2	13593.5±1274.9	0.08
Pre- and post-waking glucose concentrations			
3-hour pre-wake time-point (mg/dL)	95.0±12.0	99.0±16.6	0.29
3-hour pre-wake average (mg/dL)	93.7±12.3	96.1±14.2	0.32
2-hour pre-wake time-point (mg/dL)	94.5±12.4	96.1±15.0	0.43
2-hour pre-wake average (mg/dL)	93.6±12.7	95.4±13.8	0.34
1-hour pre-wake time-point (mg/dL)	93.1±11.9	96.3±11.4	0.30
1-hour average (mg/dL)	93.2±12.6	94.8±13.8	0.37
Waking time-point (mg/dL)	91.8±13.4	93.0±11.9	0.38
1-hour post-wake time-point (mg/dL)	92.1±13.1	90.3±10.7	0.97
1-hour post-wake average (mg/dL)	92.6±16.9	92.5±8.7	0.48
2-hour post-wake time-point (mg/dL)	95.6±15.1	93.2±11.8	0.25
2-hour post-wake average (mg/dL)	93.6±13.5	92.7±8.7	0.64
3-hour post-wake time-point (mg/dL)	98.5±15.8	94.9±14.7	0.61
3-hour post-wake average (mg/dL)	95.3±13.4	94.9±8.8	0.36

Data Presented as Mean±SD
p values are for comparisons between baseline and post-intervention

Table 6.8. Participant daily dietary intake at baseline and post-intervention

	Baseline	Post-intervention	p value
Average mealtime (HH:MM)	**13:30±00:40**	**14:00±00:39**	**0.02**
Average mealtime SD (HH:MM)	01:12±00:50	01:11±00:33	0.44
Calories per day	1366.0±389.4	1499.5±423.5	0.31
Calories per day SD	404.2±225.0	382.6 (55.0-374.8)	0.35
Carbohydrate (grams per day)	163.7±32.3	183.4±72.7	0.41
Carbohydrate SD (grams per day)	60.7±12.3	56.1±13.8	0.46
Protein (grams per day)	57.1±14.2	59.6±14.1	0.61
Protein SD (grams per day)	24.6±8.9	21.6±9.4	0.29
Fat (grams per day)	57.6±17.6	65.4±14.8	0.22
Fat SD (grams per day)	23.0±13.6	32.3±18.7	0.27

Data presented as Mean±SD, non-normally distributed data presented as median (lower quartile-upper quartile)
Time expressed in a 24-hour system as HH:MM=hour and minute
p values are for comparisons between baseline and post-intervention

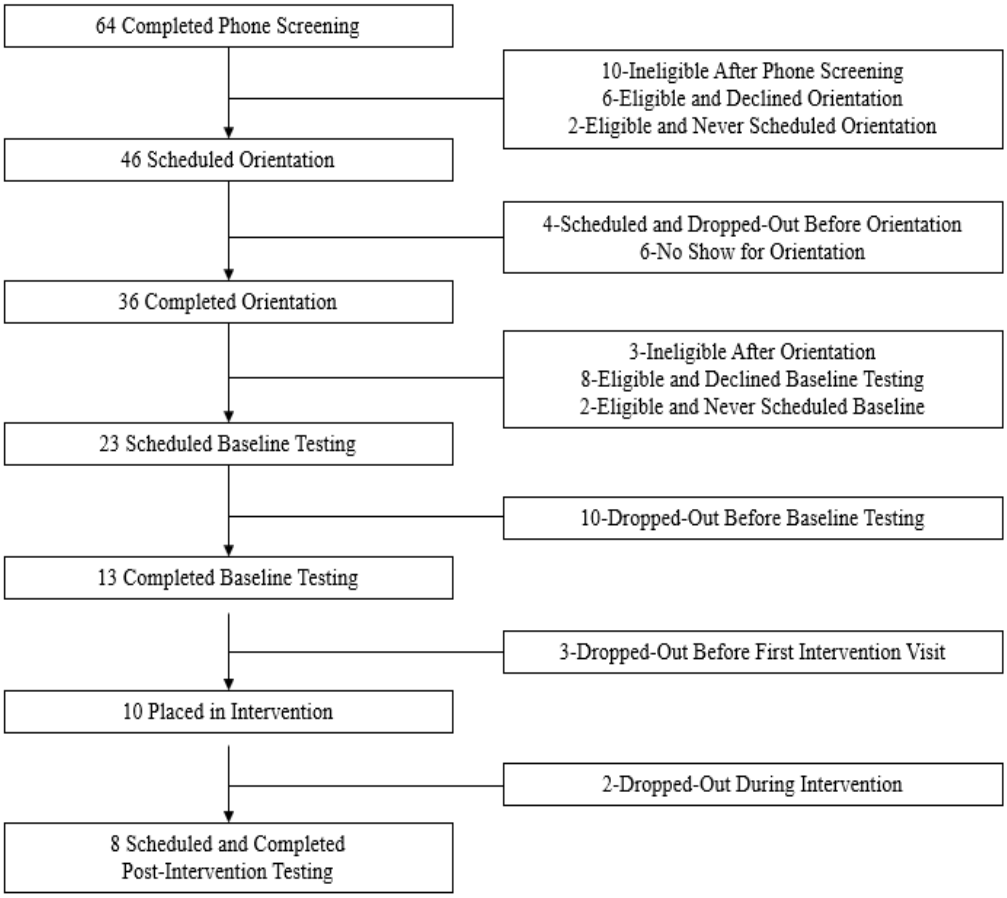

Figure 6.1. A-TEAM Study Participant Flow Diagram
The A-TEAM study participant flow diagram from initial contact at eligibility phone screening to completion of post-intervention testing.

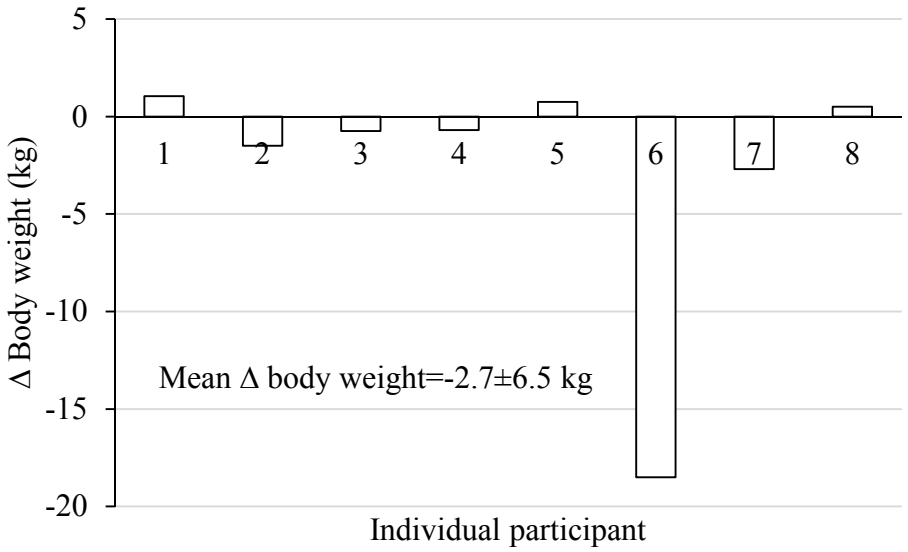

Figure 6.2. Individual Participant Δ Body Weight
Body weight changes for each individual participant from baseline to post-intervention in kilograms (kg) with mean body weight change denoted in the figure.

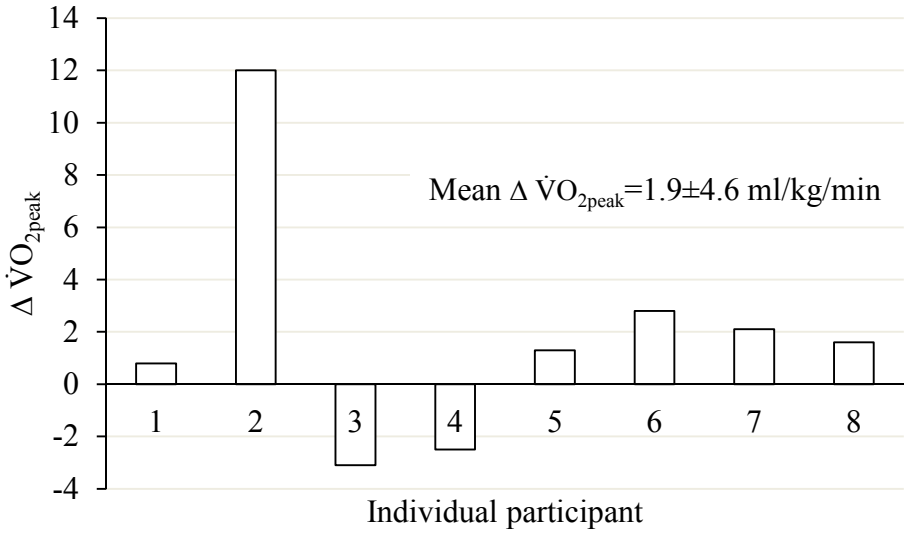

Figure 6.3. Individual Participant Δ $\dot{V}O_{2peak}$

$\dot{V}O_{2peak}$ changes for each individual participant from baseline to post-intervention in milliliters per kilogram of body weight per minute (ml/kg/min) with mean $\dot{V}O_{2peak}$ change denoted in the figure.

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CHAPTER 7

OVERALL DISCUSSION

The prevalence of adults in the United States classified as overweight or obese continues to increase (Flegal MF, Kruszon-Moran D, Carroll MD, Fryar CD, & Ogden CL, 2016; Hales CM, Carroll MD, Fryar CD, & Ogden CL, 2017). As overweight and obesity status has been linked to the development of cardiometabolic disorders, such as impaired glucose tolerance and CVD risk factors (Clinical Guideline on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report 1998; Managing Overweight and Obesity in Adults: Systematic Evidence Review from the Obesity Expert Panel 2013; Centers for Disease Control and Prevention 2017), the number of adults treated for cardiometabolic disorders will potentially trend similarly (Centers for Disease Control and Prevention 2017). Recently, glycemic variability has been introduced as a sensitive measurement of glycemic health and has been observed to be greater in overweight or obese adults compared to normal weight adults (Monnier L, Colette C, & Owens DR, 2008; Satya Krishna SV, Kota SK, & Modi KD, 2013; Suh S & Kim JH, 2015; Wang S, Lv L, Yang Y, Chen D, Liu G, Chen L, Song Y, He L, Li X, Tian H, Jia W, & Ran X, 2012; Salkind SJ, Huizenga R, Fonda SJ, Walker MS, & Vigersky RA, 2014). Additionally, greater glycemic variability has been associated with induction of increased endothelium-derived oxidative stress

commonly associated with diabetic complications and CVD (Hirsch IB & Brownlee M, 2005; Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, & Colette C, 2006; Johnson EL 2013).

Sedentary behavior and PA of varying intensities have previously been associated with metabolic function and CVD risk factors, independent of diagnosed cardiometabolic disease status, such as type 2 diabetes and CVD (Covas M-I, Elosua R, Fitó M, Alcántara M, Coca L, & Marrugat J, 2002; Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, & Owen N, 2007). Furthermore, exercise, which is a common component of daily PA, has been established as a modifiable lifestyle factor which positively influences compromised health outcomes associated with overweight or obesity status and cardiometabolic complications found in adults diagnosed with type 2 diabetes or CVD (Goodyear LJ & Kahn BB, 1998; Hawley JA 2004; Church T 2011). As findings are limited in how sedentary behavior and PA relate to glycemic variability and oxidative stress, as well as the influence of exercise on these outcomes of interest, in overweight or obese adults, without diagnosed type 2 diabetes or CVD, there remains a critical gap in the literature which remains to be addressed.

Recent evidence examining the relationship between sedentary behavior and PA with glycemic variability and oxidative stress has produced mixed findings. This could potentially be due to populations incorporated, as well as methodology utilized to specifically assess sedentary behavior and PA and glycemic variability, and delineating which biological markers of oxidative stress may be best to evaluate in their given population. Previous literature suggests that decreases in sedentary time and increases in leisure-time PA of varying intensities relate to improvements in antioxidants known to

decrease oxidative stress (Thosar SS, Johnson BD, Johnston JD, & Wallace JP, 2012). Additionally, increases in sedentary behavior have been linked to decreases in endothelium-derived nitric oxide vasodilation (Demiot C, Dignat-George F, Fortrat JO, Sabatier F, Gharib C, Larina I, Gauquelin-Koch G, Hughson R, & Custaud MA, 2007). Further, subjectively assessed low-intensity, high-intensity, and total-intensity PA EE have been shown to positively associated with antioxidant concentration (Covas M-I, Elosua R, Fitó M, Alcántara M, Coca L, & Marrugat J, 2002). However, as evidenced in previous large sample cross-sectional studies, health status may be a key regulator of the relationship between sedentary time and PA with glycemic variability, as there is currently no support to substantiate that a relationship exists between these variables in non-diabetic adults (Gude F, Díaz-Vidal P, Rúa-Pérez C, Alonso-Sampedro M, Fernández-Merino C, Rey-García J, Cadarso-Suárez C, Pazos-Couselo M, García-López JM, Gonzalez-Quintela A, 2017). Yet, in medically compromised adults diagnosed with type 1 and type 2 diabetes, sedentary time and PA of any intensity has been shown to possess a direct relationship with glycemic variability (Wadén J, Tikkanen H, Forsblom C, Fagerudd J, Pettersson-Fernholm K, Lakka T, Riska M, Groop PH, & FinnDiane Study Group, 2005; Bohn B, Herbst A, Pfeifer M, Krakow D, Zimny S, Kopp F, Melmer A, Steinacker JM, Holl RW, the DPV Initiative, 2015; Paing AC, McMillan KA, Kirk AF, Collier A, Hewitt A, & Chastin SFM 2018). Therefore, the population utilized to address the specific relationship between sedentary behavior and PA of varying intensities with glycemic variability and oxidative stress should be considered and accounted for when interpreting findings.

Even though glycemic variability has been proposed a prominent mechanism acting to induce oxidative stress, findings have been fairly constant as to what type of relationship they possess with each other in a variety of populations, including non-diabetic, type 1, and type diabetic adults (Saisho Y 2014). Previous studies examining experimentally exacerbated glucose concentration oscillations found that the higher the oscillating glucose concentrations, the greater induction of oxidative stress that occurred in both normoglycemic and type 2 diabetic adults (Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, Boemi M, & Giugliano D, 2008). Additionally, evidence has suggested the both intra-day and inter-day measures of glycemic variability exhibit strong positive associations with induction of oxidative stress in adults diagnosed with type 2 diabetes (Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, & Colette C, 2006; Di Flaviani A, Picconi F, Di Stefano P, Giordani I, Malandrucco I, Maggio P, Palazzo P, Sgreccia F, Peraldo C, Farina F, Frajese G, & Frontoni S, 2011). This is promising as intra-day and inter-day glycemic variability may present different etiologies for the induction of oxidative stress (Wentholt IME, Kulik W, Michels RPJ, Hoekstra JBL, & DeVries JH, 2008); however, whether these relationships exist in overweight or obese non-diabetic adults has yet to be elucidated.

Exercise is commonly utilized as therapeutic treatment for impaired glucose tolerance and CVD risk factors in the presence or absence of diabetes or CVD (Goodyear LJ & Kahn BB, 1998; Hawley JA 2004; Church T 2011). Exercise-induced improvements in oxidative stress has been previously studied, and chronic exercise training has been shown to decrease myeloperoxidase concentration and increase skeletal muscle nitric oxide synthase release (Richter B, Niessner A, Penka M, Grdić M, Steiner

S, Strasser B, Ziegker S, Zorn G, Maurer G, Simeon-Rudolf V, Wojta J, & Huber K, 2005; Krause M, Rodrigues-Krause J, O'Hagan C, Medlow P, Davison G, Susta D, Boreham C, Newsholme P, O'Donnell M, Murphy C, & De Vito G, 2014). However, there exists limited evidence to suggest that exercise training improves glycemic variability in adults without diagnosed type 2 diabetes (Tereda T, Friesen A, Chahal BS, Bell GJ, McCargar LJ, & Boulé NG, 2013; Van Dijk J-W, Manders RJ, Canfora EE, van Mechelen WV, Hartgens F, Stehouwer CD & van Loon LJC, 2015). Yet, evidence has suggested that, regardless of modality, that exercise training improves glycemic variability in type 2 diabetic adults, if moderate intensity during exercise is achieved (Karstoft K, Winding K, Knudsen SH, Nielsen JS, Thomsen C, Pedersen BK, & Solomon TPJ. 2013; Francois ME, Durrer C, Pistawka KJ, Halperin FA, Chang C, & Little JP, 2017). Therefore, findings from previous studies necessitate the further exploration as to how glycemic variability may be influenced by exercise training. Further, the less than ideal literature available regarding alterations in glycemic variability in sedentary, overweight or obese adults, potentially at an increased risk for the development of type 2 diabetes mellitus, undergoing an aerobic exercise intervention remains to be addressed.

The overall study goals of this dissertation were to (study 1) evaluate the current known relationship glycemic variability possesses with sedentary behavior and PA, and how a single bout of exercise or repeated bouts of exercise, as well as exercise training influences glycemic variability in a variety of populations, (study 2) examine the cross-sectional relationships between sedentary time and PA measures with glycemic variability and oxidative stress in non-diabetic overweight or obese adults, and (study 3) determine the influence of chronic aerobic exercise training on glycemic variability and

oxidative stress in non-diabetic overweight or obese adults. The first study was a critical review of the known literature revolving around the relationship between sedentary behavior and PA measures with, and the influence of single bout of exercise or repeated bouts of exercise and exercise training on, glycemic variability. The second study utilized data collected at baseline from the WORDS and A-TEAM prior to either study's intervention, while the third study utilized data collected from the A-TEAM study alone. The WORDS study (ClinicalTrials.gov identifier: NCT02413866) was designed to examine the effects of chronic moderate sleep restriction on body composition and energy expenditure in individuals undergoing a hypocaloric dietary weight loss program, while the A-TEAM study (ClinicalTrials.gov identifier: NCT03162991) examined the effects of a moderate-intensity aerobic treadmill-based exercise intervention on glucose concentrations utilizing CGM technology in sedentary, overweight or obese adults.

The second and third studies comprising this dissertation utilized cross-sectional and intervention study design to evaluate varying lifestyle factors, including sedentary time and PA and exercise, respectively, that potentially influence glycemic variability and oxidative stress. Specifically, the second study included a sample of adults from the WORDS and A-TEAM study (n=28) who had a fasting blood sample, and valid SenseWear and CGM data available over 5 consecutive days including 1 weekend day. In the third study, non-diabetic overweight or obese adults enrolled in the A-TEAM study (n=8) were included if they completed the intervention and had valid CGM data and a fasting blood sample at baseline and post-intervention. Sedentary time and PA measures were measured as time spent sedentary and performing LPA and MVPA, as well as EE for LPA or greater, and MVPA or greater. Glycemic variability was assessed as MAGE,

CONGA-1, CONGA-2, CONGA-4, and MODD. Oxidative stress was measured as nitric oxide and myeloperoxidase concentration, while the oxidative stress ratio was calculated as nitric oxide concentration÷myeloperoxidase concentration.

The first study found that the current literature utilizing CGM technology has demonstrated that potential relationships exist between glycemic control and glycemic variability with habitual sedentary behavior and PA. Additionally, a single bout of exercise or repeated bouts of exercise, regardless of modality, may reduce the prevalence of glycemic excursions and hyperglycemia immediately following, and over an extended period upon completion of, exercise in non-diabetic, as well as type 1 and type 2 diabetic adults. Lastly, exercise training has been suggested to improve glycemic control and glycemic variability in type 2 diabetic adults.

The second study found that there were significant relationships between PA minutes and EE with glucose concentrations, but not glycemic variability, while fasting glucose concentration was significantly associated with nitric oxide concentration, myeloperoxidase concentration, and the oxidative stress ratio. This led us to supports our claims that participation in PA, play a vital role in glycemic health, while glycemic variability is related to oxidative stress in non-diabetic overweight obese adults.

However, the third study found no observable changes in glycemic variability, but a significant decrease in myeloperoxidase concentration and improvement in the oxidative stress ratio. The results of the third study led us to conclude that the aerobic exercise training program improved oxidative stress, but not glycemic variability in non-diabetic overweight or obese or obese adults. Yet, following examination of average daily dietary intake, total daily mealtime was significantly later post-intervention compared to

baseline, which may explain no reductions in glycemic variability (Bandín C, Scheer FAJL, Luque, Ávila-Gandía V, Zamora S, Madrid JA, Gómez-Abellán P, & Garaulet M, 2015). Additionally, as exercise-induced improvements in insulin sensitivity and glucose tolerance begin to be lost within 5-10 days of cessation of exercise, the timing of CGM potentially played a role in our results (Heath GW, Gavin III JR, Hinderliter JM, Hagberb JM, Bloomfield SA, & Holloszy JO, 1983).

When considering the results from the three studies combined, there are several interesting findings and questions that arise. Specifically, population choice may be pertinent to evaluate the relationship between sedentary time and PA with glycemic variability and oxidative stress, as findings in type 1 and type 2 diabetics did not generally agree with findings in non-diabetic adults. Further, in the second study, although the findings between PA and glucose concentrations were in agreeance with previous literature, the participants in our study may have been generally too healthy and/or active to observe relationships between PA and glycemic variability and oxidative stress. This is evident as participants completing the baseline assessment for study two performed on average ~65.5 minutes of MVPA per day. Therefore, it could be assumed they were not only meeting current PA guidelines but exceeding them (Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, George SM, & Olson RD, 2018). Lastly, oxidative stress findings from the third study provided evidence that the aerobic exercise intervention was effective for decreasing oxidative stress as myeloperoxidase concentration decreased and the oxidative stress ratio improved. Yet, non-significant findings in glucose concentrations and glycemic variability suggests that the aerobic exercise intervention did not influence glycemic health. However, this led us

to wonder whether timing of CGM placement may play a significant role. Even though the fasting blood plasma sample was within the timeframe before detraining takes effect on biological markers of oxidative stress (Maeda S, Miyauchi T, Kakiyama T, Sugawara J, Iemitsu M, Irukayama-Tombe Y, Murakami H, Kumagi Y, Kuno S, & Matsuda M, 2001), placement and monitoring of the CGM was well within the timeframe of detraining effects on glucose metabolism (Heath GW, Gavin III JR, Hinderliter JM, Hagberb JM, Bloomfield SA, & Holloszy JO, 1983). Thus, when considering future studies examining the relationship between sedentary time and PA with glycemic variability and oxidative stress, as well as the influence of chronic aerobic exercise training on glycemic variability and oxidative stress, practical considerations for sample population and timing of CGM placement are warranted.

In conclusion, this dissertation found that habitual participation in PA of varying intensities are associated with glucose concentrations, but not glycemic variability, and oxidative stress in non-diabetic overweight or obese adults. Additionally, glucose concentrations and glycemic variability expressed a relationship with nitric oxide and myeloperoxidase concentrations, although opposite from what was expected. These findings suggest that PA potentially possesses a key role in glycemic health, while further exploration into the mechanisms of the relationship between oxidative stress and glycemic variability is necessary. Additionally, myeloperoxidase concentration decreased, and the oxidative stress ratio improved with exercise training, while there were no observable changes in glucose concentrations or glycemic variability. These findings lead us to believe that the exercise intervention positively influenced oxidative stress but did not impact glycemic health in non-diabetic overweight or obese adults. Yet,

findings on meal timing, as well as placement of CGM lead us to suggest that consideration for the effect of detraining and influence of other lifestyle factors, such as diet, should be addressed during study design.

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